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BLOOD VISCOSITY IN NORMAL AND DEHYDRATED PERSONS*

*GORDEN S. STARKEY, M.S., M.T. (A.S.C.P.)
Myers Clinic Hospital, Philippi, West Virginia

From a review of the literature for the past ten years it appears that very little work has been done, especially in this country, on blood viscosity. Some studies indicate that blood viscosity can be determined very accurately and that the test is very sensitive. It would seem that further study of this test is warranted to better establish normals as well as to show changes under various conditions. We have been interested to see whether there is any change in blood viscosity in varying states of hydration. This paper deals with blood viscosities done on sixty-one healthy young and middle aged adults and five individuals in mild dehydration.

Materials and Methods

The Hellige Viscometer No. 1200 was used throughout this study. The directions[1] accompanying this instrument were followed with the exception that a 3:2 oxalate mixture was used as the anticoagulant in place of heparin. This anticoagulant, prepared after the manner of Heller et al[2] has been found by Evans[3] to be as good as and more convenient than heparin for use with the Hellige Viscometer.

The copper sulfate method for determining whole blood and plasma specific gravities was used.[4] A line chart was used to determine hemoglobin, hematocrit and total plasma protein.

Sixty-one apparently normal persons were studied as follows: Blood was obtained by venipuncture with minimum constriction and placed in the oxalate tube and immediately mixed by gently tilting and rotating the tube.

* Hillkowitz Memorial Award. Read Annual Convention ASMT, Louisville, Ky., June, 1953.

The viscometer tubes were cleaned by drawing water, 95% alcohol, and ether through them by means of a water suction pump. The blood was thoroughly mixed and by the use of a bulb pipette the sample tube of the viscometer was filled. Using the same pipette the whole blood specific gravity was determined. The reference tube was filled with distilled water and after wetting both measuring capillaries with blood and water, repeat determinations were done until three consecutive readings were the same. The relative viscosity reading and temperature were then recorded.

The blood was then spun for 10 minutes at 2500 R.P.M. in an International centrifuge size 1 SB. The plasma was carefully removed with a bulb pipette and the relative viscosity and the specific gravity determined as for whole blood.

The absolute whole blood and plasma viscosities in Poises were then calculated, using the appropriate temperature correction factors given in the directions for use of the Hellige Viscometer. The hemoglobin, hematocrit and total plasma protein were determined.

In order to determine what effect, if any, dehydration would have on whole blood and plasma viscosities the following steps were carried out on five healthy individuals: (a) Breakfast as usual. (b) At 9:00 A.M. a urine specimen was collected for specific gravity and at that time a red blood count and hemoglobin were done on capillary blood obtained from the finger. Blood was obtained by venipuncture and placed in 3:2 oxalate tubes. (c) Blood viscosity, plasma viscosity, blood specific gravity and plasma specific gravity determinations were done as on the normal individuals. (d) Hemoglobin, hematocrit and total protein were calculated as before by the use of a line chart. (e) The individual did not eat or drink anything until the following morning and in the meantime carried on his usual activity. Blood was drawn and the same determinations as under (c) and (d) were done. In addition a urine specimen was collected for specific gravity. (f) The individual then proceeded to drink 1000 ml. of water and after one hour had elapsed the same tests were repeated. (g) The individual then drank another 1000 ml. of water and one hour later the same tests were repeated. (h) About one hour later the individual ate lunch and two hours after lunch all tests and determinations were repeated. (i) Each individual was weighed at the start, the next morning and two hours after lunch. (j) Urine specimens were collected at the time venipunctures were made except in a few instances, for one reason or another, specimens were not collected.

Results

The results of the studies are shown in Tables 1, 2 and 3. The normals for males and females are separated and given in

Tables 1 and 2, respectively. Both tables have been arranged in order of lowest to highest whole blood viscosity. Table 3 shows the findings in mild dehydration.

TABLE 1
(Males)

	*Subject	W.B.V.	P.V.	W.B.S.G.	P.S.G.	V. Hgb.	C. Hgb.	Ht.	T. P. P.	R. B. C.
1	TH	3.400	1.494	1.051	1.026	11.9	13.0	35	7.0	5.0
2	RT	3.612	1.512	1.055	1.026	13.9	12.0	41	7.0	4.2
3	JH	3.612	1.512	1.055	1.026	13.9	12.5	41	7.0	4.5
4	GH	3.655	1.494	1.055	1.025	14.1	15.5	42	6.7	5.3
5	GF	3.740	1.428	1.056	1.029	13.4	12.0	40	8.2	4.8
6	KS	3.827	1.360	1.058	1.025	15.6	14.0	46	6.7	5.0
7	CS	3.828	1.530	1.057	1.027	14.5	14.0	43	7.5	5.0
8	ER	3.864	1.494	1.054	1.025	13.7	13.0	41	6.7	4.6
9	CN	3.915	1.479	1.057	1.026	14.8	13.0	44	7.0	4.8
10	LA	4.032	1.494	1.058	1.026	15.2	13.0	45	7.0	5.0
11	RB	4.042	1.494	1.056	1.026	14.3	14.0	42	7.0	5.2
12	PN	4.050	1.445	1.058	1.027	15.0	12.5	44	7.5	4.0
13	GN	4.089	1.566	1.059	1.027	15.5	13.0	46	7.5	4.5
14	BW	4.140	1.530	1.058	1.027	15.0	14.5	44	7.5	5.0
15	NB	4.165	1.530	1.056	1.027	14.0	12.5	41	7.5	5.0
16	AW	4.813	1.530	1.059	1.027	15.5	13.0	46	7.5	4.6
17	JS	4.263	1.615	1.060	1.027	15.8	12.5	47	7.5	5.0
18	WW	4.263	1.530	1.062	1.028	16.7	15.0	49	7.8	5.3
19	JC	4.312	1.445	1.061	1.026	16.7	15.0	49	7.0	5.3
20	EP	4.316	1.558	1.059	1.028	15.2	14.0	45	7.8	5.0
21	JH	4.450	1.615	1.060	1.028	15.7	14.0	47	7.8	4.8
22	VO	4.524	1.530	1.061	1.027	16.4	14.0	48	7.5	5.0
23	RS	4.620	1.558	1.059	1.027	15.5	15.0	46	7.5	5.0
24	NP	4.641	1.530	1.059	1.028	15.2	13.5	45	7.8	5.0
25	EM	4.700	1.548	1.059	1.026	15.7	14.0	46	7.5	5.0
26	RW	4.790	1.577	1.060	1.028	15.7	15.0	47	7.8	5.3
27	GS	5.073	1.780	1.061	1.0285	16.2	15.5	48	8.0	5.5
28	HG	5.208	1.660	1.060	1.028	16.8	13.0	49	7.8	4.6
29	JD	5.220	1.615	1.058	1.029	14.4	12.5	43	8.2	4.6
30	LC	5.369	1.634	1.063	1.029	16.9	15.0	50	8.2	5.2
..	Min.	3.400	1.360	1.051	1.025	11.9	12.0	35	6.7	4.2
..	Max.	5.369	1.780	1.063	1.029	16.9	15.5	50	8.2	5.5
..	Mean	4.183	1.530	1.058	1.027	15.2	14.0	45	7.5	5.0

* W.B.V.—Whole blood viscosity; P.V.—Plasma viscosity; W.B.S.G.—Whole blood specific gravity; P.S.G.—Plasma specific gravity; V.Hgb.—Venous hemoglobin; C.Hgb.—Capillary hemoglobin; Ht.—hematocrit; T.P.P.—Total plasma protein; R.B.C.—red blood count.

Discussion

The results in Table 1 and Table 2 were at first combined but it was seen that the average whole blood viscosity in men was significantly higher than that in women. Apparently this difference is more closely related to hemoglobin than anything else. Since there is not so much difference in the average number of erythrocytes the question would arise as to the importance of size of the erythrocyte.

It is interesting to note that the average plasma viscosity for females was higher than for males. This would appear to warrant further study as it is questionable whether it is statistically significant.

It will be seen in Table 3 that the first individual drank only 1000 ml. of water and that his whole blood viscosity did not return to his base line level. It was estimated that one should lose approximately 1500 to 2000 ml. of water in 24 hours through

normal body functions. In all of the others after drinking 2000 ml. water and taking a meal their viscosity dropped below their normal. It is possible that the 2000 ml. was more than the amount lost and there resulted mild excess hydration. This idea would be supported by the very low specific gravities of urine obtained.

It can also be seen from Table 3 that the greatest change in any determination occurred in the whole blood viscosity. This could mean that heretofore unmentioned red cell plays an important role in the physiology of body water.

TABLE 2
(Females)

	*Subject	W.B.V.	P.V.	W.B.S.G.	P.S.G.	V. Hgb.	C. Hgb.	Ht.	T. P. P.	R. B. C.
1	JR	3.360	1.596	1.051	1.026	11.9	10.5	37	7.0	4.2
2	RT	3.485	1.428	1.056	1.028	13.8	11.5	41	7.8	4.5
3	AD	3.485	1.615	1.052	1.028	11.8	12.0	35	7.8	4.2
4	CJ	3.528	1.558	1.052	1.026	12.4	11.5	37	7.0	4.5
5	AG	3.570	1.512	1.058	1.028	14.8	10.5	44	7.8	4.3
6	JC	3.612	1.558	1.053	1.028	12.2	12.0	36	7.8	4.5
7	DL	3.612	1.640	1.054	1.029	12.5	11.0	37	8.2	4.0
8	SW	3.612	1.558	1.052	1.028	11.8	12.5	35	7.8	4.5
9	LH	3.652	1.558	1.056	1.026	14.3	12.5	42	7.0	4.5
10	PC	3.655	1.360	1.054	1.027	13.1	11.5	39	7.5	4.3
11	JW	3.696	1.558	1.054	1.028	12.8	13.0	38	7.8	4.5
12	PM	3.741	1.700	1.057	1.029	14.2	11.5	42	7.8	4.5
13	RG	3.780	1.576	1.054	1.028	12.8	12.5	38	7.8	4.3
14	AF	3.780	1.558	1.055	1.027	13.6	13.0	40	7.5	5.0
15	SP	3.780	1.445	1.057	1.026	14.8	14.0	44	7.0	5.0
16	PS	3.818	1.640	1.056	1.028	13.8	12.5	41	7.8	4.5
17	JP	3.825	1.494	1.054	1.026	13.4	14.0	40	7.0	5.0
18	BL	3.827	1.691	1.057	1.027	14.5	12.0	43	7.5	4.7
19	MM	3.828	1.653	1.056	1.027	14.0	12.5	41	7.5	4.8
20	BM	3.864	1.680	1.053	1.027	12.6	11.5	37	7.5	4.3
21	AB	3.948	1.512	1.058	1.026	15.3	13.0	45	7.0	4.8
22	MM	3.962	1.533	1.051	1.026	11.9	11.5	35	7.0	5.0
23	CB	3.995	1.530	1.058	1.029	14.4	12.5	43	8.2	4.5
24	GC	4.002	1.530	1.058	1.027	15.0	12.0	44	7.5	4.5
25	BS	4.032	1.848	1.052	1.028	11.8	12.0	35	7.8	4.7
26	NT	4.032	1.640	1.056	1.028	13.8	12.0	41	7.8	4.5
27	JD	4.048	1.530	1.058	1.028	14.8	12.0	44	7.8	4.5
28	JH	4.089	1.602	1.052	1.027	12.0	11.0	36	7.5	4.6
29	JT	4.165	1.596	1.058	1.028	14.8	12.0	44	7.8	4.5
30	BM	4.500	1.691	1.057	1.027	14.5	11.5	43	7.5	4.2
31	CP	5.400	1.740	1.059	1.027	15.5	14.0	46	7.5	5.0
..	Min.	3.360	1.360	1.051	1.026	11.8	10.5	35	7.0	4.0
..	Max.	5.400	1.848	1.059	1.029	15.5	14.0	46	8.2	5.0
..	Mean	3.818	1.558	1.056	1.027	13.8	12.0	41	7.5	4.5

* W.B.V.—Whole blood viscosity; P.V.—Plasma viscosity; W.B.S.G.—Whole blood specific gravity; P.S.G.—Plasma specific gravity; V.Hgb.—Venous hemoglobin; C.Hgb.—Capillary hemoglobin; Ht.—Hematocrit; T.P.P.—Total plasma protein; R.B.C.—Red blood count.

It is felt that further study of viscosity may show that whole blood viscosity can be used as a basis for an index of dehydration.

Conclusions

The average whole blood viscosity in males is 4.183. The average whole blood viscosity in females is 3.818. The average plasma viscosity in males is 1.530. The average plasma viscosity in females is 1.558. The red cell apparently plays an important role in the process of dehydration. Whole blood viscosity determinations may be useful in fluid balance studies.

TABLE 3

Individual 1 (MO)

*	W.P.V.	P.V.	W.B.S.G.	P.S.G.	V. Hgb.	Ht.	T.P.P.	U.S.G.	WT.
A	3.915	1.479	1.057	1.026	14.3	44	7.0	ND	163
B	5.220	1.602	1.059	1.027	15.5	46	7.5	1.025	161
C	4.590	1.602	1.059	1.027	15.5	46	7.5	ND	ND
D	4.312	1.584	1.058	1.026	15.2	45	7.0	ND	ND
E	4.156	1.496	1.058	1.025	15.6	46	6.7	1.015	163
					Individual 2 (CP)				
A	4.698	1.740	1.059	1.027	15.5	46	7.5	1.019	175
B	5.394	1.740	1.060	1.027	15.8	47	7.5	1.021	170
C	4.611	1.653	1.061	1.027	16.4	48	7.5	1.030	ND
D	4.504	1.653	1.061	1.027	16.4	48	7.5	1.001	ND
E	4.524	1.653	1.060	1.027	15.8	47	7.5	ND	175
					Individual 3 (EM)				
A	4.069	1.548	1.059	1.026	15.6	46	7.0	1.024	185
B	4.698	1.634	1.061	1.027	16.4	48	7.5	1.028	180
C	3.828	1.548	1.058	1.026	15.2	45	7.0	1.019	ND
D	3.698	1.548	1.057	1.026	14.8	44	7.0	1.002	ND
E	3.698	1.548	1.057	1.026	14.8	44	7.0	ND	185
					Individual 4 (GS)				
A	5.073	1.780	1.061	1.028	16.4	43	8.0	1.013	149
B	5.429	1.780	1.063	1.029	16.9	45	8.2	1.030	145
C	5.518	1.865	1.062	1.029	16.4	46	8.2	1.023	ND
D	4.891	1.735	1.061	1.028	16.4	45	8.0	1.006	ND
E	4.628	1.646	1.061	1.027	16.4	46	7.5	ND	149
					Individual 5 (BM)				
A	4.005	1.691	1.057	1.027	14.4	43	7.5	ND	208
B	4.320	1.800	1.058	1.027	15.2	45	7.5	1.016	204
C	4.500	1.890	1.059	1.027	15.5	46	7.5	1.006	ND
D	4.590	1.800	1.059	1.028	15.2	45	7.8	1.004	ND
E	3.870	1.710	1.059	1.027	15.5	46	7.5	1.003	206

*A—Normal; B—24 hour fasting; C—1 hour after 1000 ml. water, except in Individual 1 (MO) which was after 500 ml water; D—1 hour after additional 1000 ml. water, except in Individual 1 (MO) which was after additional 500 ml. water; E—2 hours after lunch; W.B.V.—Whole blood viscosity; P.V.—Plasma viscosity; W.B.S.G.—Whole blood specific gravity; P.S.G.—Plasma specific gravity; V.Hgb.—Venous hemoglobin; Ht.—hematocrit; T.P.P.—Total plasma protein; U.S.G.—Urine specific gravity; WT.—Weight in pounds.

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A SUMMARY OF TWO YEARS' EXPERIENCE WITH VIRUS COMPLEMENT FIXATION TESTS IN RELATION TO HOSPITAL AND CLINIC PATIENTS

HARRY B. HARDING, M.D., OPAL E. HEPLER, M.D.
NATHALIE SCHMIDT, M.S., AND EDNA MURMANN, M.S.

Chicago, Ill.

Introduction

Methods for the diagnosis of bacterial infections are very well established. This focuses attention upon the lack of widespread application of technics now developed for the routine diagnosis of viral and rickettsial diseases in the general hospital or public health laboratory. The authors' experience with such methods prompts this report since it is felt that persons who are contemplating the use of routine viral and rickettsial complement fixation tests may be interested in how others have established such tests in their laboratories.

Methods

The usual sheep cell—rabbit amboceptor—guinea pig complement hemolytic system was used. The amount of complement was 2 units. Both complement and amboceptor were titrated daily. The antigens were obtained from three well-known sources* and used in the dilutions recommended by the manufacturers. The controls which are accepted as standard for these determinations were employed, and were exposed to the same conditions as the test proper. Water bath fixation at 37°C for 80 minutes was adopted for the tests while the titrations were incubated according to previously published schedules.[1,2,3]

Experimental Studies

As a preliminary study to familiarize ourselves with viral and rickettsial complement fixation technics, it was thought wise to test the sera of clinic patients chosen at random. At the time tests were made these patients were symptomatically free of acute viral and rickettsial diseases. Each serum was tested routinely against the following antigens: lymphogranuloma venereum, psittacosis, influenza A, influenza B, influenza FM_1, mumps, eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, lymphocytic choriomeningitis, Japanese B encephalitis, Q fever, spotted fever group antigen, and typhus group antigen.

As the study progressed, it was decided that a compilation of the residual levels of viral and richettsial antibodies found in the

From the Departments of Bacteriology and Pathology of Northwestern University Medical School, Chicago, Illinois.

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clinic population group would be worthwhile. This would provide us with a means of checking the reproducibility of our results, and give us a base line of titer levels in a large population group against which interpretation of results obtained from hospital patients with clinical symptoms might be interpreted.

Table I presents a summary of the results obtained in this study.

In the central section of this table, the numbers in the top horizontal row are the reciprocals of the titers for which totals are given in the vertical columns. Each number of each vertical column represents the sum of the determinations where the highest titer was found to be the one represented by the figure at the top of each column. A minimum of 2 plus fixation was recorded as positive for each titer under consideration. The mean positive titers were calculated by first converting the titer to its proper concentration figure, then multiplying by the total number of positives at this titer. The sum of the concentration figures thus obtained was divided by the total of positive tests shown for each antigen.

Table I

A Summary of Results in a Virus and Rickettsial Complement Fixation Survey of Clinic Patients by Titers

Antigens	TITERS										Total No. Sera Giving Pos. Titers	Total No. Sera Tested	Mean Pos. Titer
	2	4	8	16	32	64	128	256	512	1024			
Lympho. Ven.	18	7	9	4	11	5	1	2	1	1	59	275	1-4.6
Psittacosis	10	12	12	3	13	5	1	2	1	1	60	275	1-3.9
Influenza A	50	38	27	11	8	5					139	287	1-3.6
Influenza B	44	32	47	11	13	6		1			154	273	1-4.2
Influenza FM$_1$	54	32	26	6	4						122	210	1-3.1
Mumps	26	13	6	4	1		2				52	296	1-3.0
Eastern Equine Enceph.	2	1	1								4	326	1-2.9
Western Equine Enceph.	7	2									9	292	1-2.3
St. Louis Enceph.	4										4	326	1-2.0
Lympho. Chorio.	6	1									7	326	1-2.2
Q Fever		1									1	168	1-4.0
Jap B Enceph.											0	187	...
Spotted Fever Group Antigen	3										3	187	1-2.0
Typhus Group Antigen		1									1	156	1-4.0

As soon as the results for each series of tests were completed, a history form was presented to each patient and a separate questionnaire given to his physician. From the information obtained in this manner Table II was compiled which shows the relation of the history of illness to the type of antibody found in specimens taken from each patient.

After nine months' experience in the performance of these tests with sera from clinic patients picked at random, it was decided to start testing, on a diagnostic basis, sera of patients from Northwestern University's affiliated hospitals. Each serum was first screened against all the antigens listed above in dilutions of 1:2,

1:4, 1:8, and 1:32 with the exception of lymphogranuloma venereum, psittacosis, influenza A, influenza B, influenza FM_1, and mumps antigens. The serum was routinely diluted from 1:2 through 1:64 for these latter antigens in two fold steps. Sera which were found positive in the screening procedure were tested against the reacting antigen in a second procedure in twofold dilutions until the end point was determined. Antigens from two sources were used whenever possible. After the first test was made on the acute phase specimen, it was stored in the icebox until the convalescent phase specimen was received. The two specimens then were tested simultaneously against identical antigen dilutions and hemolytic systems on the same day.

Table II

The Relationship of the History of Illness to the Type of Residual Complement-Fixing Antibody From Clinic Patients

Types of Antibodies Found	Total No. Tests	No. Sera Giving Pos. Titers*	History of Illness		No History of Illness Found
			Recent	Past	
Lympho. Ven.	275	59	1	1	57
Psittacosis	276	60	1		59
Influenza A	287	139	6	25	108
Influenza B	273	154	5	30	119
Influenza FM_1	219	122	5	25	92
Mumps	296	52		26	26
Eastern Equine Enceph.	326	4			4
Western Equine Enceph.	292	9			9
St. Louis Enceph.	326	4			4
Lympho. Chorio.	326	7			7
Q Fever	168	1			1
Jap B Enceph.	187				
Spotted Fever Group Antigen	187	3			3
Typhus Group Antigen	156	1			1

* By "Positive" is meant positive results regardless of titer.

Table III presents the over-all positive findings (not necessarily diagnostic findings) obtained from hospital patients. All of these individuals were acutely ill and febrile at the time the first serum specimen was drawn. Of these specimens, at least 250 sera were examined against all antigens listed except rabies. Additional sera were examined against lymphogranuloma venereum, Q fever, typhus, spotted fever group antigen, and typhus group antigen. The apparent discrepancy between the specific and the group rickettsial antigens in occurrence of positive findings is due to the fact that these sets of sera are not comparable. Of the sera examined with group antigens, many of them were not tested with specific antigens; while among the tests using specific antigens, there were many additional sera which were not included among those tested with group antigens. The first of these group antigens should react with sera containing antibodies against Rocky Mountain spotted fever or rickettsialpox. The second group antigen will react with antibodies to epidemic typhus or murine typhus. In addition to the above examinations, 5 sera were

examined for rabies antibodies.

Table III

A Summary of Results Obtained From Virus and Rickettsial Complement Fixation Studies on Hospital Patients by Titers

Antigens	TITER										Total No. Sera Showing Pos. Titers	Total No. Sera Tested
	2	4	8	16	32	64	128	256	512	1024		
Lympho. Ven.	8	6	2	6	4	2	1	2	1		32	251
Psittacosis	9	9	3	4	3	3	1	2			34	250
Influenza A	18	23	14	13	10						78	250
Influenza B	14	22	14	15	16	7					88	250
Influenza FM1	21	18	12	7	1	1					50	250
Mumps	19	7	13	10	7	3					59	250
Eastern Equine Enceph.	12	3									15	250
Western Equine Enceph.	5	3	3								11	250
St. Louis Enceph.	2	1	1								4	250
Lympho. Chorio.	1	2									3	250
Rickettsialpox	2				1						3	250
Spotted Fever	1										1	250
Q Fever	4	2	1								7	320
Typhus		2									2	255
Rabies											0	5
Jap B Enceph.	3										3	250
Spotted Fever Group Antigen		1		1							2	270
Typhus Group Antigen		1									1	270

In Tables IV, V, VI, and VII are presented the detailed results from 14 cases (other than those in Table III) in which an actual diagnosis was reached by the use of the complement fixation technic. Of these cases, 7 were found to react to the mumps virus antigen, 2 to the antigen of lymphogranuloma venereum, 3 to the psittacosis virus antigen, and 2 to the antigens of influenza viruses.

While it is realized that the report of a positive complement fixation test in a titer of 1:2 may be of doubtful significance, it was felt desirable here to include such findings for the sake of completeness.

Discussion

The number of positive titer responses to lymphogranuloma venereum, psittacosis, the influenzas, and mumps antigens is far greater than for the other antigens represented in this study. This is true both for the clinic group (Table I) as well as for the hospital patients (Table III). The only instances in which positive serologic diagnoses were made were for this same series of diseases (Tables IV, V, VI, and VII). It is interesting to note that in no single instance was a serologic diagnosis of an arthropod-borne encephalitis made nor was a diagnosis forthcoming for any disease caused by the classical rickettsiae. There can be little doubt that subclinical or abortive infections have been caused by these latter agents as observed in this study, however, because of the number of positive titer responses found. Some of these, eastern equine encephalitis, western equine encephalitis, and St. Louis encephalitis antigens (Table III), for

example, were fixed in titers of 1:4 and 1:8. According to Casals and Palacios,[2] titers of this magnitude can be taken as evidence of past infection. The information in Table II would support this contention. Of 275 sera listed in Table II which were tested against lymphogranuloma venereum antigen, only 2 patients whose sera were thus tested gave a history of previous illness (one recent infection and one past infection). The sera of both of these individuals gave positive titers of 1:256 with lymphogranuloma venereum antigen (see Table I), whereas the patient whose serum gave a response to the same antigen in a titer of 1:1024 (Table I) is represented in Table II as one of the 57 individuals in whom no history of illness with this agent could be obtained. Though this patient did have a positive Frei test he was not ill at the time and is in good health at present. He was admitted to the clinic for the removal of an epithelioma of the lip. It is well known that lymphogranuloma venereum is characterized by prolonged coexistence of infection and immunity.[4]

Table IV

Cases of Mumps Virus Infection Where Diagnosis Was Confirmed by Complement Fixation Tests

Patient	Sex	Age	Historical Summary	Clinical Diagnosis	RESULTS OF C-F TESTS		
					Antigen	Acute Titer	Convalescent Titer
JB	F	5	Typical mumps followed in 10 days by stiff neck, headache, fever, nausea and v., CSF lymphocytosis.	Encephalitis	Mumps	32	128
GC	M	7	Headache, stiff neck, fever, swollen glands in neck on right.	Meningitis	Mumps	4	16
VH	F	26	Headache, stiff neck, fever, CSF lymphocytosis.	Possible Polio	Mumps	16	64
					Influenza A	16	8
					Influenza B	64	32
VJ	M	32	Headache, fever, stiff neck, CSF lymphocytosis.	Meningitis	Mumps	Neg.	8, 16
					Influenza A	2	4, 4
					Influenza B	64	64, ?
					Influenza FM_1	2	4, Ne
HK	F	31	Headache, fever, stiff neck, CSF lymphocytosis.	Possible Polio	Mumps	8	32
					Influenza A	16	32
					Influenza B	32	32
					Influenza FM_1	16	16
					Murine Typhus	2	2
FF	M	23	Typical parotid mumps.	Mumps	Mumps	8	256
					Influenza B	4	4
DC	F	22	Typical parotid mumps.	Mumps	Mumps	2	64
					Influenza FM_1	16	32
					Influenza B	4	4

Whether immunity survives the disappearance of the virus has not been determined. Certainly no outward signs of illness were found in this man, though no attempts at isolation of virus were made. While it was not surprising to find a few individuals in the Chicago area with sera showing low residual titers against Q fever antigen,[5] it was surprising to discover an individual with a 4 plus fixation against rickettsialpox antigen (Table III) in a

Table V

Cases of Lymphogranuloma Venereum Virus Infection Where Diagnosis Was Confirmed by Complement Fixation Tests

Patient	Sex	Age	Historical Summary	Clinical Diagnosis	RESULTS OF C-F TESTS		
					Antigen	Acute Titer	Convalescent Titer
LH	F	19	Headache, fever, stiff neck. Exposure. Pos. Frei test CSF lymphocytosis.	Meningitis	Lympho. ven.	32	256
					Psittacosis	32	128
					Influenza A	32	4
					Influenza B	16	32
					Mumps	4	4
RW	M	33	Fever, pain, rectal granuloma. Exposure Pos. Frei test	Possible lymphogranuloma	Lympho. ven.	16	256
					Psittacosis	32	256
					Influenza A	4	Neg.
					Influenza B	2	Neg.

titer of 1:32. This person's serum did not develop a rise in titer and her illness was eventually explained on another basis. The interesting thing about her case was the fact that she had lived her entire life in the Midwest and had never been to the Atlantic seaboard. While it is possible that this is a non-specific result, it is unlikely, since only two other positive reactions appeared in the 250 specimens examined. (Table III) Could it be that rickettsialpox does occur in the Midwest and to date has gone unrecognized? On the basis of one such finding one may only speculate.

Of 4463 separate viral complement fixation tests on the sera of hospital patients who were acutely ill at the time the first specimen was examined, those from 14 patients (108 tests) yielded results which were interpreted subsequently as giving positive confirmatory evidence of virus infection. Considering the fact that one or more of these interpretations may be in error, these data do not show a high yield of positive results. Other workers, however, have reported similar results from such studies.[6] Gauld,[7] in 1947, found 272 positive specimens from 8367 viral diagnostic tests performed at the laboratories of the Army Medical School. These statistics should not cause too great discouragement, however, for the authors have found that of 1200 stools examined at the Evanston Hospital, Evanston, Illinois, during the last two years, in only 34 instances were pathogenic bacterial organisms found. Whether or not the implied analogy is valid may be questioned, but at least it does indicate that the ratio of positive data obtained to the total number of tests run is small even for bacteriological examinations. The laboratory methods for bacteriological infections can certainly be considered as far more simple to perform and to interpret than those for virus diseases.

The interpretation of any laboratory test is of utmost importance. When the tests involved are indirect ones, such as reported here, evaluation of results becomes the critical issue. In

Table VI

Cases of Psittacosis Virus Infection Where Diagnosis Was Confirmed by Complement Fixation Tests

Patient	Sex	Age	Historical Summary	Clinical Diagnosis	RESULTS OF C-F TESTS		
					Antigen	Acute Titer	Convalescent Titer
JC	M	12	Headache, fever, stiff neck, Lymphocytosis Neg. Frei test.	Meningitis	Psittacosis	32	128
					Lympho. Ven.	32	64
MJC	F	34	Fever, muscle ache, pains in chest, rales in chest, Neg. X-ray From turkey farm.	Acute upper respiratory infection	Psittacosis		
					Direct C-F	2	32
					Indirect C-F	16	32
FK	M	35	Acute atypical pneumonia. Pet shop worker. Sick parakeets X-ray typical.	Psittacosis	Lympho. Ven.	32	32
					Psittacosis	32	128
					Influenza A	4	4
					Influenza B	4	8
					Influenza FM_1	2	2
					Mumps	2	2

these studies it has been found necessary to interpret the results for physicians by written report, since the majority of doctors are not qualified to make their own interpretations. The "fourfold rule"[8] has been consistently employed. In examining Table IV it will be seen that in all instances the clinical entities involved could be caused by mumps virus.[9] Furthermore, in every case a fourfold or better rise in titer against mumps antigen was recorded. These results follow the general rules regarding interpretation as laid down by Enders.[10] The antigen employed contained both the "S" and "V" components.[11] The case of meningitis (Table V) reported as due to the virus of lymphogranuloma venereum deserves comment. Cases of meningoencephalitis caused by the agent of this disease have been reported by Sabin

Table VII

Cases of Influenza Virus Infection Where Diagnosis Was Confirmed by Complement Fixation Tests

Patient	Sex	Age	Historical Summary	Clinical Diagnosis	RESULTS OF C-F TESTS		
					Antigen	Acute Titer	Convalescent Titer
CK	M	29	Retrobulbar pain, backache, respiratory symptoms, fever.	Influenza	Influenza A	4	32
					Influenza B	Neg.	32
					Influenza FM_1	Neg.	4
					Eastern Equine Eceph.	4	4
ROW	M	22	Aches and pains, fever, acute upper respiratory infection.	Influenza	Influenza A	4	4
					Influenza B	8	32
					Influenza FM_1	4	8
					Mumps	Neg.	2
					Psittacosis	Neg.	2

and Airing.[12] The individual involved in this report was a colored female in whom no primary lesion was found but who gave an ample history of exposure.

No history of exposure could be obtained for the 12-year-old male whose meningitis is recorded as caused by the agent of psittacosis in Table VI. In this case no pulmonary lesions were demonstrated by X-ray. In the absence of any exposure to the virus of lymphogranuloma venereum and in the presence of a negative Frei test, the writers were at a loss to interpret the findings in any other way. The child in question had been quite active and could have come in contact with the agent of psittacosis in the environment of his play area. It is well known that the agent of this disease involves the central nervous system[4] as manifested by symptoms of encephalitis, encephalomyelitis, and neuritis of the facial, trigeminal, and optic nerves.

SUMMARY

1. The results obtained from the following viral complement fixation studies on the sera of hospital and clinic patients are reported:
   a. A summary of the data obtained from determining the residual levels of viral and rickettsial antibodies in the serum of clinic patients who were not ill at the time specimens were examined.
   b. The problems of interpretation which arose during the application of these tests to sera obtained from acutely ill hospital patients.
2. The experiences cover a two-year period.
3. The methods employed have been briefly reviewed.

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QUICKER BACTERIOLOGICAL RESULTS

R. H. WEAVER, Ph.D.

Bacteriology Department, University of Kentucky, Lexington, Ky.

In many medical laboratories bacteriological work is of secondary importance as compared to chemical, serological, and haemotological work not because bacteriological results are unimportant but because of certain difficulties which have been inherent in bacteriological procedures.

The most important of these difficulties is that the time required to obtain results is too long. Unfortunately bacteria cannot be identified by their morphological characteristics alone. The necessity of including cultural and biochemical characteristics has made identification a time-requiring procedure. Cultures need to have time to grow and to produce biochemical reactions. Chemical, serological and haemotological tests can be carried out in a few minutes, or a few hours at most, and the physician can be given at least a tentative report.

The second difficulty is that bacteriological tests are expensive. Technics have been so involved as to require highly trained personnel to perform them, the equipment and materials required have been expensive, and the amount of labor involved has been great. This is especially true in smaller laboratories where the volume of work is small. Many procedures are carried out so infrequently that sterile equipment, reagents and media have to be prepared fresh every time that they are needed.

More and more attempts are being made to develop new technics that will avoid some of these difficulties. Unfortunately many of these technics, developed in research laboratories, are insufficiently known in laboratories where so-called routine work is done. Actually, every medical technologist should look upon himself or herself as a research worker. The application of these technics to the practical problems that arise can only be done by the worker who is in contact with the problems. This review has been prepared in the hope of stimulating experimentation on the utilization of some of these technics.

Attempts to achieve quicker and cheaper bacteriological results have followed three lines of attack or combinations thereof. The first is the use of complex media that will allow the simultaneous determination of several characteristics of an organism. All bacteriologists are familiar with double sugar media, triple sugar media, etc. Such media are useful in that they reduce the labor involved in the identification of an organism but they do not necessarily reduce the total time which must elapse before the identification can be made. In addition, due to the complexity of the reactions involved, the results obtained are frequently not as reliable as those obtained from a series of simpler media.

The second line of attack is the development of quick or rapid technics which give results in a short period of time. The third is the development of microtechnics that require smaller amounts of material and less bulky and expensive equipment and frequently reduce the necessity of preparing fresh materials, and of using strictly sterile technic. Many of the microtechnics are, at the same time, quick or rapid technics. Some of those that have been developed are crude and are useful only as screening technics, others are at least as sensitive as the standard macrotechnics.

Koch[24] was, perhaps, the first to employ a microtechnic when he used slide cultures with semisolid media and observed growth microscopically. The "little plate method" reported by Frost[16] in 1921 was essentially a rapid microtechnic. In it a small quantity of milk was mixed with a small quantity of sterile nutrient agar on a slide which was then incubated in a moist chamber for 4-16 hours. The preparation was then dried and stained and the colonies were counted microscopically. Many modifications of this procedure for the examination of various food products have been reported. Perhaps slide culture technics such as those to be reported later for the growth of the tuberculosis organism are a development from this procedure.

In 1889, Beijerinck[3] used the auxanogram for biochemical studies on yeasts. He placed small amounts of powdered test sugars on the surfaces of heavily inoculated poured agar plates. Growth occurred in the sections of the medium into which utilizable sugars dissolved and diffused. Some of the recent microtechnics appear to be adaptations of this procedure.

Lindner,[25] in 1905, grew broth cultures in hanging drops on the under sides of coverglasses suspended over hollow ground slides, a procedure which was modified by Henneberg[20] in 1934.

Bronfenbrenner and Schlesinger,[6] in 1919, developed a micromethod for the use of fermentation tests for the identification of coliform bacteria which is very similar to some that have been in use much more recently. Drops of a substrate containing agar, indicator and fermentable substance were placed in a petri dish with a sterile capillary pipette. They were heavily inoculated and a piece of filter paper soaked in NaOH was placed in the dish to absorb CO_2.

A system of rapid microtechnics that have been developed in our laboratory appears to be applicable in many situations for the reduction of the time required to obtain bacteriological results and for the reduction of costs through savings in time and materials.

The principles involved in the techniques include the use of small tubes and large inocula which will allow the organisms to start growing without appreciable lag phases and to produce, in short periods of time, sufficient concentrations of biochemical

products to be detectable if sensitive reagents are used. All conditions, of course, need to be optimum. The best medium available for the organism must be used. To obtain results in the shortest possible time the tubes should be preheated before inoculation and incubation should be in the water bath, since the temperature of the medium is more rapidly adjusted in the water bath than in the hot air incubator. The use of cultures for inocula that are in the logarithmic period of growth shortens materially the time required for obtaining positive results. If the biochemical reaction to be tested for is one that is produced by an adaptive enzyme, more rapid results will be obtained if the inoculum is from a medium that contains a substrate on which the enzyme will have been developed. This is not necessary for accurate results, however, if a slightly longer incubation period is used. When these microtechnics are used on colonies from primary isolation plates it is difficult to obtain the optimum sized inoculum. This, however, does not affect the final results, but only the incubation time required. The objection has been raised that colonies on primary isolation plates are frequently impure cultures from which inaccurate results may be obtained. To avoid this source of error a plate may be streaked simultaneously from the colony as a check for purity. This will allow, in most cases, the saving of at least 24 hours that would be necessary for secondary plating for the purification of the cultures. Although our work is customarily done with sterile technics, when the microtechnics are used under optimum conditions absolute sterility is not essential; tubes and media do not have to be sterilized and cotton plugs are not necessary. Contaminants cannot develop rapidly enough to influence the results of the tests.

While most of our work has been done with the enteric bacteria, it is possible to adapt the technics to other types of organisms.

The first microtechnic which we developed was one for indole.[1] One ml quantities of medium are used in 10 x 75 mm tubes. A medium containing 1 percent tryptone and 0.3 percent beef extract has been found to be satisfactory, although quicker results can be obtained with a medium containing tryptophane. The media are adjusted to pH 7.4. Kovac's reagent is used for detection of the indole. The medium on which the cultures to be used for the inoculum are grown does not appear to influence the test materially. Indole tests have been obtained in from 6 minutes to 2 hours. Only two cultures have been found that will not give a strongly postive test in 2 hours. They required from 3 to 4 hours.

For the fermentation tests, 5 x 50 mm tubes containing 0.15 ml quantities of medium are used.[18] The medium that has been selected for use with enteric bacteria contains 7.5 percent Bacto beef heart infusion, 1.0 percent proteose peptone, 0.1 percent

KH_2PO_4, 0.5 percent NaCl and 5 percent of the carbohydrate to be tested that has been sterilized by filtration. A combination indicator containing bromcresol purple and cresol red is used. It has been found necessary to make the basic medium sugar free by inoculation with a culture of **Aerobacter aerogenes**, since otherwise cultures of **Aerobacter aerogenes** will produce gas in the basic medium without any added carbohydrate. **A. aerogenes** will not produce gas in the same basic medium by the usual macrotechnic. This shows that the microtechnic is more sensitive than the macrotechnic. After inoculation the tubes are capped with a 3 mm layer of plain 1 percent agar to detect gas formation.

With enteric bacteria, fermentation of glucose may be obtained in from 15 minutes to 2 hours. With a light inoculum the time may be slightly extended. Disaccharides require about 50 percent more time for fermentation.

McCollum[26] was able to adapt the test to the study of streptococci using a basal medium containing 1 percent tryptose, 1 percent proteose peptone #3, 0.5 percent NaCl, 0.1 percent KH_2PO_4, and phenol red as an indicator. The medium was adjusted to pH 8.0 before sterilization. The final medium consisted of 3 parts of the basal medium, 1 part of sterile fresh horse serum and 1 part of a 20 percent solution of the test carbohydrate sterilized by filtration. Fermentations were detectable within 2 hours under optimum conditions.

With tests that are more quantitative than qualitative such as tests for hydrogen sulfide and acetylmethylcarbinol production, the microtechnics are more sensitive than the macrotechnics. By careful standardization of the procedures it is possible to get results that correlate well with those of the macro-tests or to get results that indicate production of these products by many cultures that give negative results with the macrotechnics, according to the desires of the investigator.

For the hydrogen sulfide production tests, a medium consisting of a 2.0 percent solution of thiopeptone in distilled water is used.[29] It is dispensed in 0.8 ml amounts in 10 x 75 mm tubes, and a lead acetate paper is used as an indicator. With coliform organisms, results which agree well with those of various macrotechnics can be obtained with a 45 minute incubation period. It must be recognized, however, that no two macrotechnics will give identical results with a series of coliform cultures. Longer incubation periods with the microtechnic increases the sensitivity well beyond that of the macrotechnics.

For the detection of acetylmethylcarbinol production, a medium consisting of 0.7 percent glucose, 1 percent trypticase and 0.5 percent NaCl, in filtered meat infusion, is used.[15] It is tubed in 0.5 ml amounts. Incubation is at 30 C for 90 minutes. Modified Barritt's reagents are used with the tubes being shaken for 5 sec.

after the addition of each reagent. The results are read after 30 minutes incubation in the water bath. Again it is possible to get results that are too sensitive by increasing the incubation periods or by increasing the time of shaking the tubes.

Reduction of nitrates to nitrites can be detected by the use of the usual nitrite reagents after an incubation period of 15 minutes using a medium containing 1 percent peptone, 0.3 percent beef extract and 0.1 percent potassium nitrate.[2] Results are identical with those with the macrotechnic.

For the detection of citrate utilization, a medium containing 0.5 percent sodium citrate, 0.25 KH_2PO_4, 0.05 percent K_2HPO_4 and 0.4 percent agar is used in 1.5 ml amounts.[19] The heavy inoculum is carefully placed on the surface. As the enzyme system is adaptive the inoculum comes best from a medium containing citrate. Alkali production as evidence of citrate utilization is detected by adding a drop of bromthymol blue solution after an incubation period of 90 minutes, negative reactions being checked after an additional 2 hours.

It should be emphasized that the microtechnics developed in our laboratory are not screening technics but are as sensitive as or more sensitive than the corresponding macrotechnics. Some of the technics to be discussed later in this review may require less equipment and labor with a large series of cultures but, in some cases, at least, they are less sensitive.

Modifications of our microtechnics include those of Greene *et al*[17] for detecting motility. They were able to detect motility microscopically following 15 to 30 minutes incubation in small quantities of nutrient broth, and macroscopically after 90 to 120 minutes incubation in tubes of semisolid agar following stab-inoculation.

Hoyt[21] used similar technics for the demonstration of urease production and indole production except that he had the media prepared in tablet form by Boyle and Co. of Los Angeles. The urea tablets contained urea, buffers, yeast extract and phenol red according to the formula of Stuart, Van Stratum and Rustigian.[39] The indole tablets contained tryptophane, NaCl and dicalcium phosphate. For the tests, 1 tablet was dissolved in 1 ml of distilled water. The technics of carrying out the test were the same as in our microtechnics. Sterilization of the substrate was found to be unnecessary.

In 1952 Bicknell, Butt and Mattman[5] reported upon a screening procedure for enteric organisms using a microtechnic with similar principles to those that have been described. By the use of semisolid test media which they inoculated by stab inoculation they were able to detect glucose and lactose fermentation, with or without gas formation, H_2S production, urease production and motility within a period of 3½ hours. This procedure de-

tected 30 percent of the paracolons; a longer incubation time detected more of them.

McCready and Holmes[27] of the laboratories of the Massachusetts Department of Public Health have instituted a time-saving procedure by which they can isolate and identify enteric pathogens from stools in two days or less instead of the usual three or four days. For a screening procedure they fish colonies from the primary plates into tubes containing 0.3 ml amounts of lactose-sucrose phenol red broth and incubate for one to three hours in the 37 C water bath. If, when growth appears, acid production is evident, Endo plates are streaked to guard against enteric pathogens being obscured by contaminating acid formers. If no acid production is evident, hanging drop examinations for motility and agglutination tests are performed. Confirmatory carbohydrate broths are then inoculated, still on the same day on which the primary colonies were picked. They also recommend that where bacteriologists who are experienced in the field are handling large numbers of specimens microtechnics also be used for confirmatory tests. According to their schedule, at 9 A.M. they inoculate lactose-sucrose phenol red broth with the suspected colony, inserting a lead acetate paper. At 11 A.M. they determine motility and set up agglutination tests. At 12 Noon they read the results of the agglutination tests and inoculate urea medium and confirmatory carbohydrate media in 0.3 ml amounts by means of a capillary pipette. They use glucose agar to detect gas production and broths for the other carbohydrates. At 4 P.M. they read the results of the biochemical tests and do an indole test on the lactose-sucrose broth, thus completing the identification from the colony in 7 hours.

Numerous procedures for the quick determination of urease production, especially for the identification of *Proteus*, have been described. The method of Stuart, van Stratum and Rustigian[39] was to inoculate 3 loopfuls (2 mm loop) from an agar slant culture into a modification of the medium of Rustigian and Stuart[35] in which the buffer content had been reduced to 0.1 that of the original medium, shake the tube, and incubate in a "37.5 C water bath." With this procedure they obtained positive results with *Proteus* in from 40 seconds to 60 minutes. In 1946, Christensen[11] proposed a medium which would allow the detection of urease production by *Proteus* in 1-6 hours, and by paracolons, *Aerobacter*, and paracolon intermediates usually within 24 hours.

Roland, Bourbon and Szturm[34] recommended a buffered medium containing urea, tryptophane and phenol red for differentiating *Proteus* and paracolons from *Salmonella* and *Shigella*. In this medium, *Proteus* and certain paracolons could be detected in 2-4 hours by urea decomposition and differential media could be inoculated. An indole test after 24 hours served as confirmation.

Elek[14] described a method which he claimed to be better than that of Stuart, van Stratum and Rustigian[39] for the identification of *Proteus* in that it would detect a much smaller amount of urea destruction, maintained a high buffer content as a protection against false results, and could be used with a smaller inoculum. He criticized Christensen's[11] medium because it contained an alternative source of nitrogen, which, although it allowed the identification of other organisms such as paracolons, introduced a source of error with respect to *Proteus*. Elek's medium is a buffered urea solution made up in ammonia-free distilled water. It does not need to be sterilized. Five tenths ml amounts are inoculated with a straight needle from agar slant cultures and are incubated at 37 for 3 hours. Urea decomposition is detected by Nesslerization.

Singer[36] proposed the use of a medium containing tryptone, urea and a combination of indicators. This medium could be used for the detection of urease and indole production and was useful for the differentiation of *Paracolobactrum, Proteus, Salmonella* and *Shigella*.

A number of ingenious and useful microtechnics and rapid technics have been developed, using solid culture media.

Negroni and Daglio,[30] in 1949, made a study of the assimilation of sugars and nitrogen sources by yeasts by Beijerinck's[3] auxanographic method, the Oxford cylinder method, the use of filter-paper discs, and an agar cup method, obtaining the best results with the Oxford cylinder method.

Knox[23] devised a screening plate for the rapid identification of pathogens and the rapid elimination of non-pathogens in the routine bacteriological examination of feces. The medium is poured into a petri dish in two parts. Part A, consisting of lead acetate, sodium thiosulfate and full strength tryptic heart broth in 2 percent agar, is poured with the petri dish at a slight angle. When this has set, part B, consisting of sodium desoxycholate, lactose, neutral red and half strength tryptic heart broth in 2 percent agar, is poured with the petri dish flat. The plate is inoculated by streaking heavily across the center of the plate at right angles to the division between part A and part B, and lightly over the remainder of the plate. Two strips of blotting paper, one soaked in 50 percent mannitol and the other in 50 percent sucrose, are placed on the heavily inoculated part of the plate on medium B. A glass coverslip is placed on the heavily inoculated part of the plate on medium A and another on medium B close to the strip of blotting paper that was soaked in mannitol solution. After overnight incubation the medium indicates spreading of *Proteus*, pigment production of *Pseudomonas*, sucrose, lactose, and mannitol fermentation, gas production from mannitol under the one cover glass and H_2S production under the other. The purity of cultures may be readily checked on the part

of the plate that was lightly streaked and single colonies are available for inspection of colony form and for serological investigation.

In a later paper, Cook and Knox[12] reported excellent results in the rapid recognition of presumptive pathogens and the early elimination of non-pathogens in the examination of over 1,000 colonies using the screening plate method of Knox,[22] supplemented by a rapid urea test and an indole test. In the urea test they used Christensen's[11] medium with incubation for 1 hour in a 37 C water bath. The indole test was similar to the one reported by Arnold and Weaver,[1] with incubation for 1 hour.

Svejcar and Sterzl[41] have prepared a modification of Knox's[23] method in which they use an agar containing urea, glucose, bromthymol blue, lead acetate and sodium thiosulfate as medium A and Endo agar as medium B. A coverslip is placed over part A to show CO_2 and H_2S production. Pieces of paper soaked in sucrose and mannitol solutions are placed on part B. They claim to be able to differentiate *B. coli, Proteus,* salmonellae and shigellae in 24 hours.

Karakasevic[22] modified the procedure of Knox[23] by adding another medium to the petri dish, for the detection of urease, and using Andrade's indicator instead of neutral red. He also added the sugar solutions by loopfuls to the B part of the plate instead of adding them in strips of blotting paper. Each was covered with a coverglass. He carried out detailed biochemical investigations of the suspected colonies in small tubes containing semisolid yeast extract agar.

In 1951, Dingle and Solomons[13] proposed a micromethod for screening large numbers of cultures for their enzymatic activities. They used the method primarily on fungi. Cylinders, up to 40, made of porcelain or glass, were sterilized, placed on a thin polyvinyl chloride sheet in a petri dish, and filled with 0.1 ml amounts of agar aseptically from a hot water jacketed microburette. These were then inoculated and incubated in a moist chamber. For estimating the metabolic products, the plugs were transferred to a glass sheet by extrusion with a glass rod. A suitably buffered 1.5 percent agar solution containing the substrate to be tested and salicylanilide to prevent further growth was poured over the plugs and the glass sheet was incubated for 24 hours and developed with a suitable reagent. By this method formation of α amylase and galacturonase was followed daily. Kojic acid production was shown by development with ferric chloride. Antibiotics were assayed and effects of nitrogen sources on growth were determined.

Spaur and Wynne[38] determined fermentation by embedding strips of filter paper soaked in 50 percent solutions of carbohydrates in an agar with indicator in a petri dish. They inoculated

the plates at right angles to the strips of filter paper, and placed sections of coverglasses over the glucose paper to detect gas formation.

Snyder, Donnelly, and Nix[37] have been able to incorporate nutrients, test substances, and, in some cases, reagents in 3/8 to 1/2 inch bioassay discs. The discs are dry heat sterilized, moistened with sterile or freshly prepared solutions, dried 15-20 minutes in the open under an infra-red lamp, and stored in any satisfactory receptacle. These discs are placed on the surface of heavily inoculated agar plates, using a medium containing 1.2 percent agar and 0.5 percent NaCl. By this method acid and gas production from carbohydrates, citrate utilization, H_2S production, indole formation, hydrolysis of urea and production of acetylmethyl carbinol may be determined. All results except gas formation have been found to be very reliable. Results are usually obtained within 3 hours.

Chapman[10] was able to determine up to 16 biochemical reactions on a single agar plate by the use of sterile glass tubes pushed 1 mm into the agar. One or two drops of appropriate reagents were placed in each tube and the results were observed. Fermentations, reduction of triphenyltetrazolium chloride, gelatinolysis, etc., were determined.

Turner-Graff[42] has developed a method that is, perhaps, less subject to error and more adjustable to different purposes than many of the methods in which agar media are used. Small droplets of agar are pipetted aseptically onto the bottom of a petri dish. From 8-30 of these plaques may be placed in the bottom of a dish. As they set almost instantly, the dish may then be turned over and other plaques placed on the top of the dish. Different reagents and test materials may be placed in the plaques and various reactions may be determined. Cylinders may be sealed into the plaques for the application of reagents. As the plaques are totally separated there is no danger of diffusion from one plaque affecting the results with neighboring plaques.

Poetschke and Bommer[32] have developed a system of observations of living organisms in liquids and in agar film cultures under the phase microscope that makes it possible to go much farther in identifying organisms from microscopic observation in a short period of time.

Many applications of rapid technics and microtechnics for specific purposes may be found in the literature.

Stuart,[40] in our laboratory, was able to adapt our rapid fermentation test to confirmation of positive presumptive tests in water analysis. With brilliant green lactose bile broth in small tubes, heavily inoculated, capped with agar and incubated in a 37 C water bath, it was possible to obtain positive reactions in from 15 minutes to 4 hours. Results were as reliable as those obtained

in 48 hours by the standard procedure. Chapman[9] has described a medium on which it is possible to confirm the presence of *Escherichia coli* as distinct from other coliforms due to its inability to reduce triphenyltetrazolium chloride.

The importance of rapid methods and micromethods for determining antibiotic resistance is well known. The various assay disc methods fall into this category. In addition, a few recent methods are of interest.

Mirecke[28] has described an agar plate method by which he is able to recognize 90 percent of pyogenic bacilli from pus and determine their resistance to antibiotics within 24 hours.

Walch[44] describes a method for making a bacteriological diagnosis and determining resistance to antibiotics within 4 hours. The test material is placed on an ascites agar plate and one area is covered with a coverglass. On other areas single drops of various antibiotic test solutions are added. All are covered with coverglasses. The coverglasses are sealed with vaseline. After 3-4 hours incubation the plate is examined under the phase microscope. Microcolonies of all bacteria are visible in the first area. Only colonies of bacteria that are resistant to the various antibiotics are visible in the other areas. For confirmation of diagnosis the coverglasses may be lifted and the bacteria attached to them may be stained. A similar procedure is described by Poetsche and Hanke.[33]

Vietinghoff-Scheel and Hagendorf[43] developed a plate test for the sensitivity of glucose-fermenting bacteria for antibiotics in which they used a heavily inoculated pour plate with a medium containing glucose, serum and water-blue. They punched holes in the agar and filled them with 2-drop amounts of diluted antibiotic, and incubated at 37 C, examining after 3-5 hours and 16-24 hours. The antibiotic inhibits blue staining of the medium by the bacteria.

There is no record of anyone having compared carefully the various types of rapid technics and microtechnics. From what information is available it appears that technics which use liquid media, such as those developed in our laboratory, are more sensitive and more useful for final tests. They are less subject to error except for errors due to the difficulty of being certain of pure cultures from primary isolation. This error can be avoided by simultaneously streaking on an agar medium. Some of the technics with agar can be carried out with less labor, especially when a large number of cultures are to be investigated, but these technics appear to be more useful as screening technics. Detection of gas formation from carbohydrates is not always reliable and reactions which depend upon change in pH must be watched very carefully because of the speed of reversal of pH changes in a thin film of medium.

Some work has been done in an attempt to develop more rapid methods for the detection of specific bacterial pathogens. Perhaps the most extensive is the work on tuberculosis. Many attempts have been made to develop slide culture methods. Early results were disappointing, but more recent results have been promising. Berry and Lowry[4] were able to obtain positive slide cultures in 3.5 days as compared to 37.4 days when they used the common technic with Petragnani's medium. The number of positives was somewhat less, indicating that their procedure would serve best as a screening procedure. Oeding[31] found a slide culture technic to be inferior to the use of Lowenstein's medium when small numbers of bacilli were present, but was able to obtain uniformly positive results with the slide method when large numbers of bacilli were present. Recently Brueck and Buddingh[7] have been able to consistently demonstrate tuberculosis organisms in the yolk sacs of embryonated eggs 4-6 days after inoculation with tubercular material.

Cameron and Castles[8] have reported a procedure that will save from 64 to 88 hours in the cultural identification of the gonococcus. From oxidase positive colonies they rinse off the reagents from the organisms with sterile broth and inoculate into 0.2 ml amounts of sterile sugar broth base. If gram-stained preparations from the sugar broth base are satisfactory, fermentation tests are run with dextrose, maltose, sucrose and levulose by a micromethod. Glucose is fermented in 6 hours. Contaminants always ferment in less than 6 hours. Negative results at 6 hours may be checked in 24 hours.

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THE ISOLATION AND IDENTIFICATION OF DERMATOPHYTES*

LEANOR D. HALEY, Ph.D., M.T. (A.S.C.P.),
MARY STONEROD, M.T. (A.S.C.P.)

Department of Microbiology, Yale University School of Medicine
New Haven, Connecticut

The diagnostic medical mycology laboratory is requested to isolate etiological agents of dermatophytoses more often than those fungi responsible for the systemic mycoses. Although the actual technic of collecting material to be planted and the isolation of the dermatophytes is much simplier than that involved in the study of the systemic mycoses, the actual identification of these organisms is often more difficult than the identification of any of the other human pathogenic fungi. There are several reasons for this, among them being that many of the dermatophytes, especially the Trichophyton genus, resemble one another so closely microscopically as well as grossly that only an expert can tell one species from another and even then he may have difficulty. Often it is only by use of special media that these fungi are differentiated. The use of animals in the final identification can not be made as they are when one is attempting to identify those fungi giving rise to systemic infections.

It is the purpose of this paper to discuss in some detail methods of collecting materials for study and the isolation and identification of the organisms giving rise to the tineas. It is hoped by the authors that this paper will be of aid to those technologists whose training has not included much mycology but who, nevertheless, are expected to isolate and identify species of the dermatophytes.

The dermatophytes are parasites of animal and man, living in superficial keratinized parts of the body as the nails, hair and skin. They do not have, however, the ability to give rise to systemic infections. Among those diseases caused by dermatophytes are athlete's foot or tinea pedis, tinea corporis, tinea capitis, tinea cruris and onychomycosis. These organisms fall into three genera, Microsporum, Epidermophyton and Trichophyton. It is well to remember that the species of Microsporum have the ability to invade hair and skin, *Epidermophyton floccosum* (only one species in this genus) invades nails and skin, and Trichophyton species can invade hair, nails and skin.

Microsporum audouini and *M. canis* are the only species of this genus that are of real importance in this country. Infections due to *M. gypseum* are rarely seen. The epidemic form of ringworm of the scalp is due to *M. audouini*. Ninety per cent of the cases of tinea

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capitis are of this type; the remaining ten per cent of these cases are usually due to *M. canis*. The clinical picture of ringworm of the scalp due to these fungi resemble one another closely and it is only by use of the laboratory that the physician can be sure with which organism he is dealing. Ringworm of the glabrous skin is usually due to *M. canis, Trichophyton mentagrophytes* or *T. rubrum*. At the present time there are about twelve recognized species of Trichophyton although it is possible to find many more listed in the medical literature. Most of these, however, have been reduced to synonymy. In southern New England *T. rubrum* has been isolated frequently in cases of tinea pedis while *T. mentagrophytes* is often found to be more common in other parts of the country. *Trichophyton rubrum, T. mentagrophytes* and *Candida albicans* are probably the chief etiological agents in fungus infections of the nails. Although tinea capitis is usually due to *M. audouini* and *M. canis* there are areas of the country where *T. tonsurans* is found to be the etiological agent. Indeed, there is evidence that this fungus, which gives rise to a more serious form of the disease, is gradually spreading from the southern part of the U. S. to the eastern areas. We have isolated *T. tonsurans* on several occasions from patients seen in the dermatology clinic of the Grace-New Haven Hospital.

Tinea barbae is a chronic fungus infection of the face and neck. There are two clinical forms recognized—the superficial type which resembles tinea corporis and has as its etiological agents the same species of fungi that invade the glabrous skin, and the deep suppurative type of infection. We have recently seen three patients with the latter type of infection from which *Trichophyton faviforme* was isolated. It is interesting to note that a fungus infection was not suspected in any one of these patients when first seen in the Surgery Clinic.

Tinea cruris is a fungus infection of the groin and perianal region and has as its chief etiological agent *Epidermophyton floccosum* although *T. rubrum* or other species of Trichophyton may give rise to this infection. Clinically the lesions are very characteristic for this infection, but the laboratory is needed when the physician is considering seborrheic eczema, contact dermatitis or moniliasis.

COLLECTION OF MATERIAL: It is necessary to epilate under a Wood's light hairs suspected of being invaded by fungi. This light is obtained by the utilization of ultraviolet wave-lengths of 3650 Å, which is obtained by passing through a Wood's filter composed of glass with nickel oxide. Becker and Obermayer[1] state that "the Corex filter of red- or blue-purple glass (No. 586 Corning Glass Works) may be substituted for the Wood filter." Although it is possible to make one of these lights yourself they can be easily purchased at moderate prices.* Usually those hairs which have been

* Strobelite Company, New York City; Westinghouse Electric and Manufacturing Company, Long Island City, New York.

infected will be seen to fluoresce under this light although it must be noted that not all fungi will cause this fluorescence. The Microsporum species fluoresce as do *T. tonsurans, T. schoenleini. Trichophyton mentagrophytes* and *T. rubum* do not fluoresce hair. Hairs that are infected will be broken, sometimes close to the scalp and can be easily removed from the hair follicle with forceps. As the hairs are epilated they may be placed in a sterile petri dish or between two slides that have been wrapped together in paper and sterlized. We prefer the slides for we are able to write a few brief notes about the patient on the paper wrapping and they also allow for filing of material for future reference without taking up much space. In traveling from clinic to clinic or ward to ward it is very easy to carry 10 to 15 sets of slides whereas one can only carry a few petri dishes.

The collection of skin scrapings is a little more difficult. The skin must be carefully wiped with an alcohol sponge to remove surface contaminants, then, using a sterile scalpel or a Bard-Parker knife blade and handle, gently scrape the periphery of the lesions. Since lesions of ringworm of the skin heal from the inside out and spread peripherally, the fungus will be found living in the border of the lesions—not in the center where healing is taking place. These scrapings can be collected directly into a sterile petri dish or between sterile glass slides.

The technique of collecting nail scrapings is a tedious one both for the technologist and the patient. It is important to remember that the nail is composed of layers of highly cornified epithelial cells although it is thin at the point of origin where it is being formed and pushed forward. Since there is no way of telling at what level within the nail the fungus is growing, it is necessary to shave the nail gently down to the layer directly above the nail bed. By examining and culturing these shavings it is possible to isolate the fungus, whereas if one simply clipped the end of the nail with scissors and cultured this clipping there is a strong possibility that fungus studies may be negative. If, however, a whole nail is sent to the laboratory for study it is necessary to shave this nail by holding it firmly with tweezers and then gently shaving it with a scalpel.

To isolate fungi from the feet one must first take care to cleanse the area to be examined with an alcohol sponge for contaminating fungi and bacteria are present on the feet. If there is no clinical evidence of tinea pedis, scrape the fourth interspace of both feet and any calluses present, for it is here that a fungus may often reside in a quiescent state. It would seem that the crushing of the little toe to the fourth toe as is done by a shoe is enough to cause moisture and maceration to develop and, thereby, give rise to an excellent medium for a fungus. Also examine the instep for any area of slightly discolored scales

of epithelium. Remember that individuals can carry fungi on their feet asymptomatically yet develop serious "id" reactions; therefore, don't refuse to scrape a patient's feet because there is no evidence of fissuring, cracking, maceration, oozing, or even itching. When collecting the scrapings don't take dead scales which so often rest on the surface of a lesion—remove these and gently scrape the epithelial layers between the toes. If fissures are present scrape the periphery of these sites; the deep erythematous tissues will not yield fungus at this time nor is it likely that the patient will permit you to touch this area too roughly. Again scrapings may be collected in a sterile petri dish or between glass slides.

EXAMINING AND PLANTING OF MATERIAL: Hairs should be examined in a drop of ten to forty per cent potassium hydroxide by placing one of the epilated hairs in a drop of this clearing agent and then gently heating the slide being careful NOT to bring the preparation to a boil. When examined under low and then high power it will be noted that the fungus appears as spores arranged in a mosaic sheath surrounding the hair shaft or that the spores are in linear arrangement outside the hair shaft (ectothrix) or inside the hair (endothrix). Microsporum species do not invade the hair and have their spores in mosaic arrangement. Trichophyton species have their spores in linear arrangement and may be inside or outside of the hair. Thus, although it may be possible to determine the species of fungus present in tinea capitis it is possible to determine which genus is present by means of the potassium hydroxide preparation. Cultures are needed to determine the species. To each of two slants of Sabouraud's dextrose agar* place three to four hairs at short intervals of space. Incubate these cultures at room temperature. Usually growth will be detected within four to five days but it will take about seven to ten days before growth is ample to permit identification. Cultures are held three weeks before being reported negative for fungi. Cultures made from scrapings of patients who have been using fungistatic ointments may be negative or exhibit delayed growth. If bacterial contaminants have interfered in the culturing of organisms it is possible to go back to the original material and make new cultures. Bacteria may die over a period of several days although the fungi will not. It is also advisable to make fresh cultures of those materials yielding a positive potassium hydroxide preparation but whose cultures are negative.

To study skin and nail scrapings we examine them in potassium hydroxide preparations and with the modified Hotchkiss-McManus stain[7] as well as culture them. It has been observed

* APPENDIX.

repeatedly that the modified Hotchkiss-McManus stain may pick up a fungal element in material when the technologist has read the potassium hydroxide preparation as negative. Indeed, it cannot be over emphasized that reading potassium hydroxide preparations for the presence of fungi in skin and nail scrapings is most difficult and to a beginner can give serious problems. On the other hand, the Hotchkiss-McManus stain is not difficult to do, requires about 30 to 45 minutes to do one or twenty slides and is most rewarding as far as revealing fungi. Following preparations of slides for microscopic studies, several pieces of skin or nail scrapings are placed on each of two Sabouraud's agar slants which are then incubated at room temperature. Again cultures are kept for three weeks before discarding as negative.

If hairs or skin scrapings have been removed from the bearded area of the face and neck of a patient with lesions of a deep suppurative character one should consider the possibilities of isolating *Trichophyton faviforme* which is the species commonly associated with this type of lesion. Growth of *T. faviforme* on Sabouraud's medium is slow and scanty. Georg[5] has shown that special vitamin enriched media is necessary for satisfactory growth of this fungus as well as incubation at 37° C for primary isolation. She used a heart infusion tryptose thiamine agar.* It is also possible to use Difco Blood Agar Base to which thiamine (0.1 mg thiamine added to 100 ml melted, partially cooled agar) is added. Since these suppurative lesions also have bacteria present as secondary invaders, streptomycin (40 units per ml of medium) and penicillin (20 units per ml of medium) should be added to this medium.

CULTURAL STUDIES: *Microsporum canis* is easily identified by its pointed, rough, thick walled, multicellular macroconidia (fuseaux) in conjunction with the canary-yellow to bright orange-yellow which is developed on the back of the colony when grown on Sabouraud's medium. This fungus grows rapidly, developing dirty white aerial mycelium. It may or may not have microconidia which are of no value in identifying the organism.

M. audouini is more difficult to identify. Unlike *M. canis*, it is reluctant to develop macroconidia on Sabouraud's medium. When it does so they are usually few in number, being abortive in appearance—that is, they are long, tapering, possessing perhaps 2 to 5 cells, and are often indented. This fungus grows more slowly than does *M. canis*, possessing little aerial mycelium and usually developing an orange-brownish pigment on the back of the Sabouraud's slant.

There are several things that the technologist can do to aid in the identification of these organisms. It is a recognized fact that when cultured on rice medium[4] *M. canis* grows fluently over the surface of the rice granules while *M. audouini* will not grow on such a medium. Hazen[6] recommends culturing *M. audouini* on honey-

peptone agar with 5 mg of yeast extract per ml of medium to bring about the development of the macroconidia which are characteristic of this organism.

Epidermophyton floccosum is among the easiest of all the dermatophytes to identify when first isolated. It is characteristically powdery to velvety with a beautiful sulfur-green color. Microscopically no microconidia are seen but the large, smooth multiseptate, "snow shoe" macroconidia are characteristic. This fungus, however, is most difficult to keep in a stock culture collection for within three to four weeks sterile, white, cottony, aerial mycelium rapidly develops and it then becomes impossible to identify the fungus.

The identification of the Trichophyton species is the most difficult among the dermatophytes. Fortunately, the studies of Georg, Robbins, Lewis and Hopper as well as others have made this a much easier task for the diagnostic mycologist. *T. mentagrophytes* is usually a rapidly growing organism, powdery to granular or even cottony in appearance. When grown on Sabouraud's medium it is usually white with a yellowish-brown back. Occasionally, however, a freshly isolated strain may have a pinkish pigment in its growth with a wine-colored back. More often species of *T. mentagrophytes* develop a reddish pigment as they age. The development of this pigment causes this species of Trichophyton to become easily confused with *T. rubrum*. This latter species is usually cottony but can be powdery in appearance and, as the culture becomes older, the aerial mycelium also becomes pigmented. Microscopically it is difficult to distinguish between *T. rubrum* and *T. mentagrophytes*—a few macroconidia are developed on Sabouraud's medium and the microconidia of the two species resemble each other so closely that only an expert can differentiate them. An important diagnostic aid[2] in identifying these species is the use of corn meal dextrose agar.* When cultured on this medium *T. mentagrophytes* does not produce any pigment and at least 95% of the strains of *T. rubrum* will develop the characteristic reddish-purple color. By culturing *T. rubrum* on Difco Blood Agar Base[2] it is possible to force it to develop macroconidia which may be of some value as an aid in the identification of this organism. These macroconidia vary in length with the number of cells ranging from 3 to 10 with an average of seven. They are long, narrow, thin-walled, almost pencil-shaped.

Trichophyton tonsurans has been classified by Conant in the Crateriform group—a group characterized by having colonies with varying degrees of heaped or sunken central growth with a rough, wrinkled or folded surface. In the medical literature *T. epilans*, *T. sulfureum* and *T. Sabouraudi* were other species belonging to this same group but Georg, believing that there is not enough difference between these species to warrant them separate entities, has reduced them to variants of *T. tonsurans*. Colonies are usually

* APPENDIX

powdery to slightly cottony varying in color from creamy white to yellow to reddish-brown. Microscopically there are many microconidia borne terminally, laterally or on short sterigmata. These spores are usually larger in size than the microconidia of *T. Mentagrophytes* and *T. rubrum.*

T. schoenleini, etiological agent of favus, belongs in the faviform group of fungi as does *T. faviforme.* This group is characterized by being slow growing, heaped, folded, usually lacking serial mycelium, and often waxy in apperance. Favus is a form of ringworm of the scalp caused by *T. schoenleini, T. violaceum* or *Microsporum gypseum.* The lesions are yellowish, cup-shaped with crusts which, when removed, reveal soft, erythmatous bases. The hair is broken off close to the scalp. If the disease goes untreated it will spread over the scalp, scar tissue will develop and permanent alopecia may result. The diagnosis is usually made clinically on the presence of the yellowish scutula (concave crusts) and the very characteristic "mousy odor." The laboratory is needed to identify the species of fungus giving rise to the infection. Fortunately, favus is not an infection seen in many parts of the country. A fairly large number of cases are found in New York City where immigrants enter the country.

Acknowledgment Is Made to the Nancy Sayles Day Foundation for Assistance in This Work

APPENDIX

1. SABOURAUD'S DEXTROSE AGAR:

Dextrose	40 grams
Peptone	10 grams
Agar	35 grams
Distilled water	1000 ml

Autoclave at 15 lbs. pressure for 15 minutes and slant. It is not necessary to check the pH.

2. CORN MEAL DEXTROSE AGAR:

Yellow corn meal	62.5 grams
Distilled water	1500 ml

Heat for one hour by running in the autoclave without pressure, or at 60 C for one hour in a water bath. Filter through absorbent cotton. Bring to original volume; add 13 grams of agar per 1000 ml of broth. Distriubute in small flasks and autoclave at 15 pounds pressure for 8 minutes. Just before use add aseptically enough dextrose to the amount of 2%.

3. HEART INFUSION TRYPTOSE THIAMINE AGAR

Beef heat, infusion from	500 grams
Bacto—tryptose	10 grams
Sodium chloride	5 grams
Bacto—agar	15 grams
Thiamine hydrochloride	10 grams
Distilled water	1000 ml

Dissolve the ingredients in the water and adjust to pH 6.8 Autoclave at 121C for 10 minutes.

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SOURCES OF ERROR IN ELECTROCARDIOGRAMS

By SISTER MARY ANTONIA KLAPHEKE,
S.C.N., M.T. (A.S.C.P.), M.S.

Cardiology has become a specialty. One needs but to note the incidence of heart conditions to understand the necessity of more attention to this important branch of medicine, and no test is of more value to the cardiologist than the electrocardiogram. Its use leads to the recognition and analysis of the arrhythmias; is valuable in detecting myocardial involvement when physical signs of such structural alteration are not present, as in coronary sclerosis and thrombosis; yields information concerning the presence of myocardial changes which cannot be detected in any other way; is of value in detecting enlargement of the ventricles and in the control of the administration of digitalis and quinidine; and provides in graphic form a permanent record of the case which is invaluable for reference in future treatment.

All of this is gleaned from the reading and interpretation of the electrocardiogram, a graphic picture of the electrical voltages generated by the muscular action of the heart. Normally nerves and muscles are classified as excitable tissues, and they respond with certain propagated patterns to adequate stimuli.[1] By means of electrodes attached at pertinent points on the body, this action current of the heart can be picked up and recorded.

The typical electrocardiogram of a cardiac cycle consists of a series of waves arbitrarily designated by Einthoven as the P wave, the QRS complex, the T wave, and the U wave. Ordinarily the P, R, and T waves are upright or positive deflections, and the Q, S, and U waves are usually negative deflections.[2] Amplitude and depth of waves, length of complex, as well as prolongation of the intervals are all part of the necessary measurements of electrocardiography.

As you continue to do more and more tracings, you become familiar with the type of picture represented in each lead. This observation is purely within the range of technical work and not bordering in the least on interpretation. It is merely the recognition of a satisfactory tracing for presentation to the cardiologist.

Read before the American Society of Medical Technologists, 21st Annual Convention
Brown Hotel, Louisville, Kentucky
June 17, 1953

Frequently the negative aspect of technic is not presented: the sources of error and the means to correct or compensate for these sources of error. There is a great deal of difference between thinking that you have something that is right and knowing that it is not right and how to correct it. In this lies the difference between an automatic individual and one who understands what he is doing. The technical procedure of electrocardiography should be as accurate as that of the other procedures in the clinical laboratory. Presenting an incorrect tracing can certainly cause a misinterpretation as far as diagnosis is concerned, and improper therapy could even be instituted as a result.

There are some distortions of the typical patterns due to mechanical features. Example: the respiratory type of curve occurring in a cardiac patient with rapid breathing (Figure 1-A).

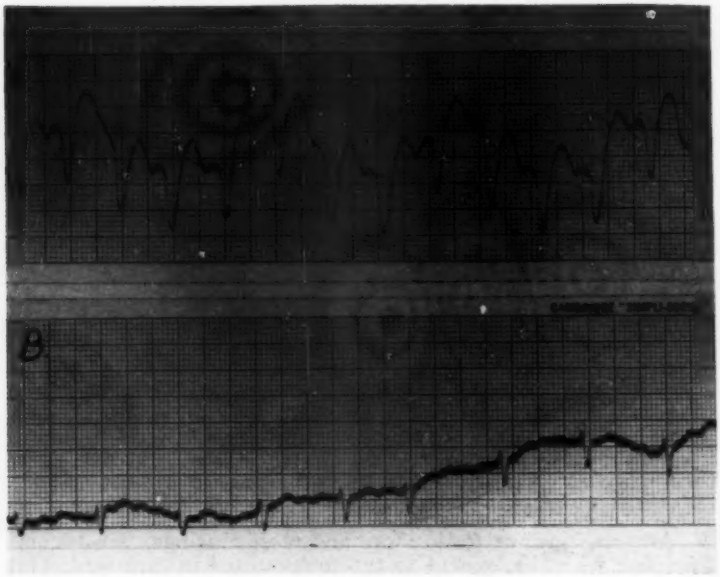

Figure 1-A. Respiratory type of curve.

Figure 1-B. Wandering string.

As the chest moves up and down, the curve will move correspondingly. The only way that this irregularity might be eradicated would be to ask the patient to hold his breath while the tracing is being made. This, of course, would be hazardous. These patients are dyspneic, and holding the breath could cause

respiratory embarrassment and even death. In this particular instance we would simply allow the curve to wander. A slight aberration will not interfere with the interpretation. It can easily be seen to be regular and respiratory in origin. This should not, however, be confused with the shift in the base line due to skin currents, polarization of electrodes, or change of voltage resistance of the skin at the point of contact (Figure 1-B). It is different. The wandering string is temporary and will right itself if a sufficiently long tracing be made. This artefact should be recognized at the time it occurs and adequate tracing made.

Each lead in the electrocardiogram has a typical pattern, and there are means by which each pattern may be checked. For instance, Lead II is the algebraic sums of Leads I and III; a Vr is the mirror image of Lead I. This should be of considerable help in recognizing the error of incorrect attachment of the lead wires. This is not a too common error, but it is not unknown to technicians of experience to have it happen occasionally. In attaching the wires to the patient, if the technician does not read the initials to make sure he has them in correctly, if he does

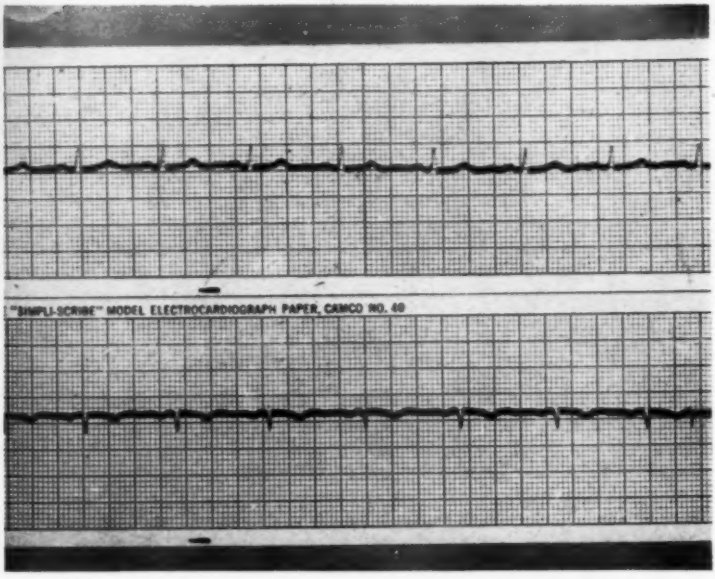

Figure 2. Mirror image resulting from an incorrect attachment of lead wires.

not make his second check by noting the color of the rubber tips, he should recognize that he has these wires twisted when the tracing is made. The error gives us an exact mirror image to Lead I and should be quickly detected (Figure 2).

Technicians are also taught to have proper tension on the string. The accepted standard is a one centimeter deflection for each millivolt introduced into the galvometric circuit. Overshooting results in abnormally high amplitude of the deflections with slurring and widening of the complexes; undershooting results in lowered voltage. Occasionally it might be necessary to reduce voltage, but again this is done with standard technic: the voltage should be reduced by half and the reduced standardization shown on the graph. Figure 3 illustrates an explicit example of this necessity. In this particular case the deflections of the chest leads before reduction of amplitude touch the top and bottom of the graph paper. It cannot be determined whether the ST segment comes off below the base line, and the exact

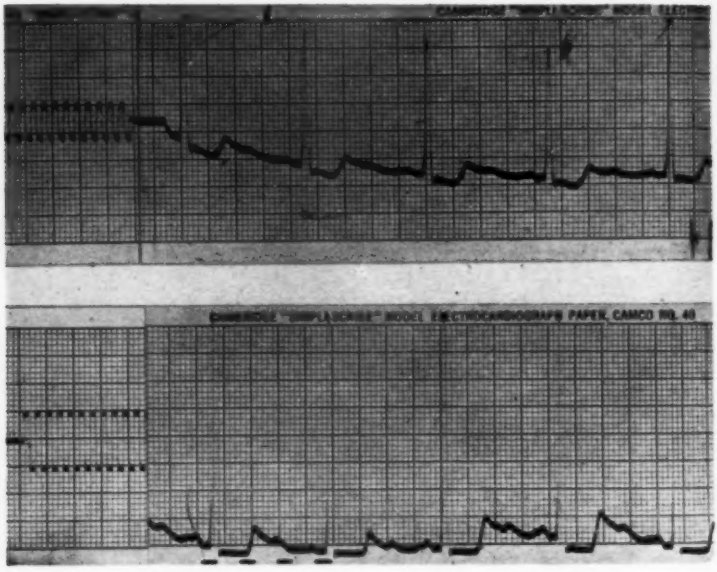

Figure 3. Example showing the necessity of reducing voltage.

pattern of the T wave is not certain. After the voltage is reduced to one-half, the ST segment comes off below the base line, and

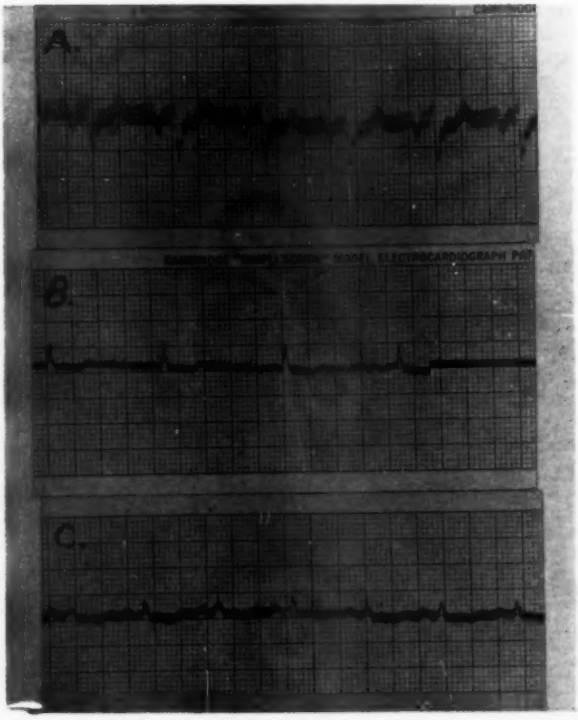

Figure 4-A. Fuzzy and broadened line.

Figure 4-B. Muscle tremor.

Figure 4-C. Alternating current.

the T wave is diphasic. Both of these features are abnormal. Significant points would have been missed had the first tracing been accepted.

Another common artefact which is seldom confusing, but which is very annoying and sometimes difficult to eliminate, is a fuzzy and broadened line (Figure 4-A). A number of things might be the cause of this (each of which must be tested): poor grounding, broken wire, profuse sweating of patient, dehydrated skin, application of electrode over bony area, poor contact between electrode and patient. Occasionally, or rather it might be said "rarely," it is the machine itself. Each of these things has

its own remedy, which is rather apparent. In this same category might be placed the type of fuzziness of the line due to muscle tremor (Figure 4-B), be it from too tight an electrode or from a neurologic condition such as Parkinsonian disease, in which the vibrations of the line are irregular in rate and not uniform in height as they are in alternating current (Figure 4-C). The loosening of the electrode in the first instance will remedy the trouble, but in the second, needless to say, the artefact cannot be eliminated. Alternating current difficulties (Figure 4-C) can be eliminated by changing electrical outlets, by detaching other electrical appliances, or by additional grounding.

Other annoying artefacts are those occurring when the patient moves (Figure 5-A) or coughs or cries (Figure 5-B). Again, simply by running the tracing an additional length, these can be compensated for.

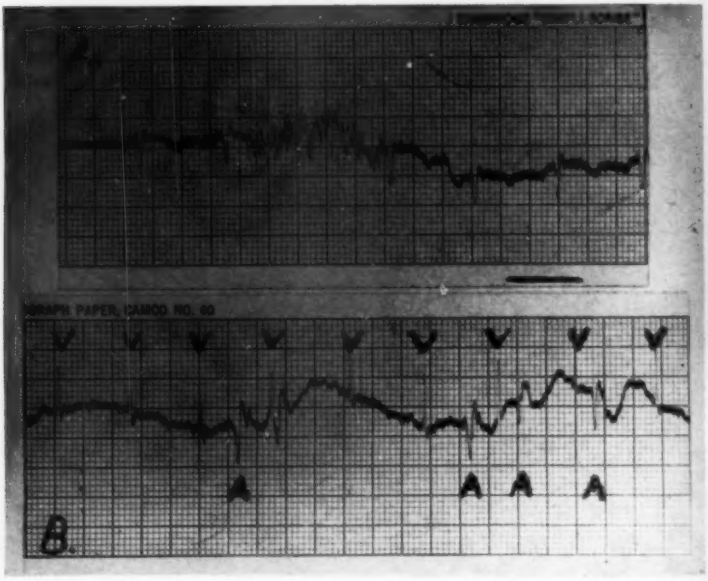

Figure 5-A. Artefact caused by movement of patient.

Figure 5-B. Artefact caused by crying of the patient.

While not too many electrocardiograms are ordered on babies, one of the sources of error in doing the chest leads on infants should be pointed out.[3] The chest area is small, care is not

always taken in applying the electrode paste, and when the large chest electrode is used, the tracing that results is identical in all six chest positions (Figure 6). The purchase of an infant electrode and the use of an alcohol sponge instead of the paste will completely eradicate the error.

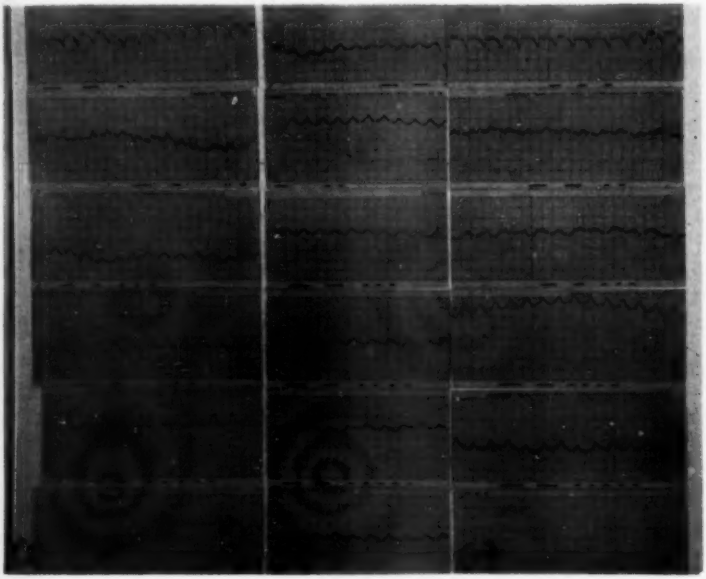

Figure 6. Tracing made on infant: (a) Using small Welch electrode. (b) Using the conventional electrode. (c) Using large Welch electrode.

In conclusion, several slides are presented which at first glance might be considered by the inexperienced as further demonstrations of artefacts, but when observed more closely, they will be recognized for what they are:

1. Ectopic beat or ventricular extrasystole (Figure 7-A)
2. Auricular flutter (Figure 7-B)
3. Diphasic P exceeding QRS complex (Figure 7-C)

Recognition of true and false artefacts comes only with diligent study. To know what can be eliminated and how to do it comes only with practical experience. Let him who is interested in presenting a satisfactory tracing to his cardiologist study and work faithfully.

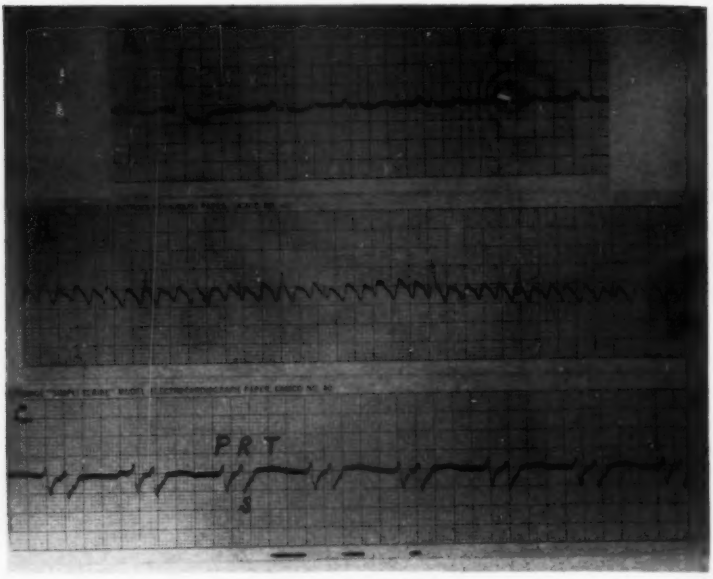

Figure 7-A. Ectopic beat.

Figure 7-B. Auricular flutter.

Figure 7-C. Diphasic P exceeding QRS complex.

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MEDICOLEGAL RESPONSIBILITY OF THE MEDICAL TECHNOLOGIST*

GEORGE E. HALL

Staff Associate, Bureau of Legal Medicine and Legislation, American Medical Association

In general, the medical technician is subject to the same medical hazards and the same legal involvements as any other person living in this complex age. There are some medicolegal problems which are somewhat peculiar to the practice of medicine and therefore of general interest to medical technicians because of their connection with the practice of medicine. Two of such problems are of more than general interest, namely: the legal status of the medical technician and the legal liability of the medical technician.

A medical technician, as I understand the term, is a person who performs various laboratory tests and procedures on blood, blood serum, urine, sputum and other body contents or by-products, the results of which tests and procedures are used or interpreted by licensed physicians in making a diagnosis. As most of you probably know, medical technicians are required to be licensed in only three or four states, none of those states being represented at this meeting. Because medical technicians are thus able to function without having to meet any specific legal standards, they must carry a twofold burden. In the first place, they must somehow establish and enforce their own standards. Secondly they must voluntarily control and limit their activities to such an extent that they do not encroach upon the practice of medicine. From what I have been able to discover it would seem that the activities of the Registry are exerting an exemplary force toward the establishment and maintenance of proper standards. And to the extent that medical technicians continue to perform their services for physicians and make no effort to hold themselves out to the public as independent diagnosticians their legal status as a very valuable assistant to the physician and hospital will not be questioned. Medical technicians must always remember that they are not the doctor, they are the doctor's employee or the hospital's employee as the case may be and as such are an integral part of the laboratory team which makes scientific medicine what it is today.

As such employee what is the medical technician's liability in the event that he is negligent in the performance of his duties and a third person is injured as a result of such negligence? It has always been a cardinal principle of American law, and of the

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English common law before that, that every person should be liable for the consequences of his own negligent and wrongful acts. In this respect medical technicians are no different from any other member of the general public. They too are liable for the consequences of their own acts. I have heard nurses and technicians express the opinion that they need not worry, the hospital or laboratory as the case might be would have to pay. Perhaps the hospital or laboratory will have to pay—but, in the eyes of the law, the medical technician is personally liable for any damages caused by his carelessness or negligence. That is something which so many employees of physicians, hospitals, laboratories and blood banks either do not realize or do not remember.

There are some situations however in which the technologist will have company so far as liability is concerned. Under our common law, things which are done for us by others are construed as being our own acts. Furthermore an employer must respond in damages for the negligent acts of its employees. Put another way, the things a medical technician does in the employ of a hospital or laboratory are really the acts of the hospital or laboratory and such hospital or laboratory is liable for any damages caused by the technician during the performance of his regular duties. Of course, if the employee were to step aside from his regular duties and perform some act which injures another, then only the employee would be liable. For example if he went out driving in the evening and caused an automobile accident the employer would not be liable. On the other hand if the technician is negligent in carrying out his regular duties in the laboratory and some injury to a third person results then your employer might be held liable for the injuries. This liability does not work any injustice to the employer for it is based upon the policy of protection of the third person and results from the consideration that it is the employer who makes it possible for the employee to cause the injury in the first place. The liability is also based on the further reason that the employer holds out his employees as competent and in effect warrants their conduct in all matters within the scope of employment. Now, what are some examples of instances in which the employer is held liable. There are two excellent cases that may be cited to illustrate this situation. (1) The National Homeopathic Hospital maintained on its premises a laboratory and employed a medical technician to perform services therein. Arrangement was made with an outside physician, who had overall charge of the operation of the laboratory. In this particular case the hospital, in the usual course of its operations, ordered a blood test on a patient preliminary to performing a blood transfusion. The technician without the presence or supervision of the physician, made the test and re-

ported directly to the hospital authorities. Unfortunately the technician made an error of some kind and blood was reported as being compatible when actually it was incompatible. Death followed the transfusion which was subsequently performed. For this death the Circuit Court of Appeals for the District of Columbia held the hospital as the employer of the technician to be liable (*National Homeopathic Hospital v. Phillips* 181 F. (2d) 293 (1950)). (2) In one of the most recent cases on the subject which has come to my attention the negligence concerned a laboratory technician employed by the Mississippi Baptist Hospital. Acting on proper orders the technician took a blood sample of the deceased and of another patient on the same floor of the hospital for the purpose of determining the blood types prior to transfusion. The types were properly and correctly ascertained as type 4 for the deceased and type 2 for the other patient. In preparing the blood to be used by each patient the next day, however, the technician reversed the two labels. The result was that during surgery the next morning the deceased was transfused with type 2 rather than type 4 blood. According to the death certificate the deceased died from "acute hemolytic anemia due to blood transfusion of wrong type." Although it was found that the hospital had been in no way negligent in selecting and retaining the technician, the Supreme Court of Mississippi held the hospital to be liable for the negligent act of its employee which proximately caused the death of the deceased (*Mississippi Baptist Hospital v. Holmes* 55 So. (2d) 142 (Mississippi 1951)).

Of course the technician in each of these cases was also liable. A plaintiff will naturally sue the defendant from whom he has the best chance of collecting a judgment and hospitals with their insurance protection are usually more solvent than a medical technician employed by the hospital would be. The liability is there, however, even though the medical technician is completely broke.

The reason for emphasizing this personal liability is because of the interest in preventing negligence cases rather than in determining who is liable after some negligent act has already been done. If the technician realizes that the liability is his liability not just the liability of his employer, he will be more apt to conduct himself so as to prevent the liability from arising. Medical technicians have both a legal and a moral obligation to themselves and to their employer to do nothing which might cause injury to an innocent third person. Under the pressure of speed and overwork the medical technician can very easily make a mistake. The slightest inattention on the part of the technician can result in serums becoming interchanged, in bottles of blood becoming interchanged, in names becoming mixed up, in the wrong pilot tube being attached to a bottle of blood, in a failure to properly read a label or properly prepare a label. These are just a few

of the things that could happen and for which the technician and his employer would be liable.

In the April 18 issue of The Journal of the American Medical Association there appears a report of the American Medical Association's Committee on Medicolegal Problems entitled "Medicolegal Aspects of Blood Transfusion." The report was prepared under the direction of Dr. Alexander S. Wiener of New York one of the country's leading authorities in this field. Among other things he highlights the important part which medical technicians have in the practice of scientific medicine by saying "the increasing use of blood in the operating room for patients under anesthesia makes it necessary to emphasize one's complete dependence in such cases on the laboratory."

Many times one reads about the importance in all phases of scientific activities of the human factor. The medical technician is one of the human factors in today's practice of scientific medicine. The importance and responsibility of the medical technician should never be belittled.

EXFOLIATIVE CYTOLOGIC TECHNICS WITH SPECIAL CONSIDERATION OF NEWER METHODS†

CHARLOTTE M. STREET, B.S.

*Papanicolaou Cytology Laboratory**
Cornell University Medical College, New York, N. Y.

Exfoliative cytology as originally applied to the diagnosis of cancer of the uterus, required fairly simple technical procedures. Secretions from the female genital tract were spread directly on slides, fixed in a solution of equal parts of ether and 95% alcohol for fifteen minutes, and then strained.[1] During the past few years, however, the method has found a wider application in the diagnosis of malignant neoplasms of the gastro-intestinal, genito-urinary, and respiratory tracts, and of metastatic tumors of the pleura and peritoneum. These newer applications have introduced new technical problems since the accurate interpretation of exfoliated cells requires material which is sufficiently cellular, well preserved and well stained.

Sputum, being mucoid in nature and containing a high concentration of cells, may be smeared directly and fixed in the ether-alcohol solution, and therefore offers no particular problem. Since there is often delay in receiving the specimen, however, it is preferable to have it collected directly in 70% alcohol for immediate fixation of the cells and to retard the growth of bacteria and fungi.

Other body secretions which are less mucoid and less cellular present more difficulties than those from the female genital tract and sputum. First of all, there may be rather rapid degeneration of the cells due to acidity of the fluid, presence of bacteria, digestive enzymes, or other factors. This can usually be overcome by the immediate addition of an equal volume of alcohol to the specimen as soon as it is obtained, refrigeration until it can be centrifuged and the supernatant liquid discarded, and avoidance of needless delay in preparing smears.

The second problem, that of a comparatively low concentration of cells in fluids can easily be taken care of by centrifugation of the specimen. This is done for 20 to 30 minutes at about 1500 R.P.M. and smears are prepared from the sediment.

The third and perhaps the greatest problem in handling fluids is that of making the smears adhere to the slides throughout the staining process. The washing off of material, particularly in large amounts, is of course a serious matter. Obviously, no report can be given if there is insufficient material present on the slide, and it is often difficult to obtain more fluid, particularly if it is a ureteral, gastric or bronchial specimen. Considerable time has been lost by the technician in the preparation, staining and

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screening of the smears, not to mention the loss of time for the doctor and inconvenience to the patient if special procedures were necessary to obtain the specimen. Last but not least, there is always the danger of contamination, since cells which become detached from one smear may stick to another smear of an entirely different case. This may have serious consequences, as a false positive report may be given, possibly resulting in needless surgery. Recognition of contaminents is usually possible microscopically as they often lie at a higher focusing level than the original smear, but may be difficult if the smear is thick and unevenly distributed.

In order to reduce to a minimum the loss of cells and the danger of contamination, the following procedures are suggested for the preparation and staining of sediment smears. After a specimen has been centrifuged and the supernatant liquid decanted, a few drops of Mayer's albumen are mixed with the sediment to make it more cohesive. This is a procedure described in "The Cytologic Diagnosis of Cancer" by the Staff of the Vincent Memorial Laboratory at Massachusetts General Hospital,[2] and has produced good results. Next, clean slides are spread with a thin film of Mayer's albumen to provide an adhesive surface for the cells. Sediment is spread in a uniform smear on a slide, using the flat surface of another slide. Just before the smear begins to dry, the slide is immersed very gently in a fixing solution of equal parts of ether and 95% alcohol. Smears are fixed for at least one hour before straining and longer if possible.

As a further precaution against losing cells, one of the most helpful procedures we have found is to treat the smears with celloidin to give them a protective coating before straining them. The slides are transferred without drying to a 1% solution of celloidin in ether and 95% alcohol (equal parts) for about one minute. They are next placed in 95% alcohol for about 30 seconds and then run through the straining procedure as described below. The thin coating of celloidin remains on the smear until it has been stained and dehydrated, and does not seem to alter the staining reaction.

The standard Papanicolaou staining procedures[1,3] have been modified especially for the straining of sediment smears. According to the original technic, smears were stained in hematoxylin for six minutes, slightly decolorized by dipping six times in 0.25% aqueous solution of hydrochloric acid and then placed in running tap water for six minutes. It was noted that cells started to float off when the smears reached the water and continued to fall off in subsequent solutions as they were being dehydrated. In order to avoid this, a weak solution of ammonia in 70% alcohol has been substituted for the hydrochloric acid and

running water, for differentiation and bluing of the nuclei. The staining procedure now used in the Papanicolaou Cytology Laboratory is as follows:—

1. Fix smears in ether and 95% alcohol, equal parts, for a minimum of one hour.
2. Place in a 1% celloidin solution made up in ether-alcohol for 1 minute.
3. 95%, 80%, 70%, 50% alcohols, distilled water, approximately 30 seconds in each.
4. Harris hematoxylin (made without acetic acid and diluted with an equal volume of distilled water)—2 minutes.
5. Rinse gently in distilled water, then 70% alcohol.
6. Differentiate in NH_4OH, 1.5% in 70% alcohol, 1 minute.
7. Rinse in 70% alcohol, 2 changes.
8. Stain in OG-6*, 1½ minutes.
9. Rinse in 95% alcohol, 2 changes.
10. Stain in EA-65**, 1½ minutes.
11. Rinse in 95% alcohol, 3 changes.
12. Absolute alcohol—1 minute.
13. Absolute alcohol and xylol, equal parts—1 minute.
14. Xylol and mount without drying.

The standard technic using hydrochloric acid and running water is still the procedure of choice for heavy mucoid preparations such as gynecological and sputum smears, as the cytoplasm is more completely decolorized, retains its transparency and therefore takes the counterstain better. The ammonia method as just described is especially suited to urinary smears as they are thin and most prone to wash off, but it may be used for other sediment smears as well. Which ever method is used, particular care should be taken to avoid agitation of the rinsing and dehydrating solutions when transferring the slides from one solution to another. Also, the stains, alcohols, xylol, etc., should be filtered frequently to rid them of any cellular debris.

The ammonia technic is not a very recent development—it has been in use in the Papanicolaou Cytology Laboratory and other laboratories for some time—but is given here for the sake of completeness in dealing with this particular problem.

Another problem of considerable importance in the cytologic diagnosis of cancer is that of obtaining well preserved cells

*OG-6

Orange G, 0.5% solution in 95% ethyl alcohol	100 cc.
Acid phosphotungstic	0.015 gm.

**EA-65

Light Green SF 0.05% solution in 95% ethyl alcohol (yellowish)	45 cc.
Bismark Brown 0.5% solution in 95% ethyl alcohol	10 cc.
Eosin yellowish 0.5% solution in 95% ethyl alcohol (water and alcohol soluble)	45 cc.
Acid phosphotungstic	0.200 gm.
Lithium carbonate (saturated aqueous solution)	1 drop

in gastric secretions. The digestive action of the gastric enzymes and the presence of hydrochloric acid, even in small amounts, cause rapid deterioration of the cells. Even though alcohol is added immediately, the cells in some specimens show degenerative changes to such a degree that definite evaluation in regard to malignancy is impossible. It has therefore become necessary to develop a technic whereby freshly exfoliated cells from the gastric mucosa can be obtained before any destructive changes take place.

The first gastric smears studied in the Papanicolaou Cytology Laboratory were prepared from ordinary fasting aspirates which had been mixed with 95% alcohol. Some of these showed good cellular preservation which made accurate interpretation possible. On the other hand, in a large percentage of cases, cytolysis, nuclear degeneration, and the presence of retained food elements resulted in smears which were usually considered unsatisfactory for cytologic evaluation.

In order to eliminate the presence of old, degenerated cells, and food particles in gastric smears, and to obtain freshly exfoliated cells, it was decided to aspirate and discard the accumulated gastric contents, lavage the stomach with a physiologic solution, then aspirate and save the washings for cytologic examination. Various solutions were tried but Ringer's yielded the best results. It was also found by fluoroscopy that a Rehfuss tube with a bucket tip was preferable to other types since the heavy metal tip carried the tube further toward the pylorus than other kinds of aspirating tubes which tend to coil in the stomach. The lavage specimen was mixed with 95% alcohol, centrifuged and smears prepared from the sediment. The resulting smears were considerably better than those prepared from an ordinary gastric aspirate as the cells were better preserved and there was less debris present.

Although the quality of the cells found in lavage specimens was improved it was felt that the number might be increased by means of gentle mechanical irritation of the gastric mucosa, and that the freshly exfoliated cells obtained by this means would retain even more of their original character than those obtained by washing the stomach. For this purpose a gastric balloon was devised, as described by Panico, Papanicolaou and Cooper in the Journal of the American Medical Association, July 1950.[4] It consisted of a double-lumen gastric aspirating tube with a thin rubber balloon attached to the distal end. The surface of the balloon was covered with many small knots of silk suture. After the balloon was introduced into the stomach it could be inflated and moved up and down, producing gentle friction of the lining of the stomach. Many experiments were performed both as to construction of the apparatus and methods of collecting speci-

mens. The chief drawback of the balloon as originally designed was the time consumed in tying by hand about 200 knots to its surface.

The abrasive action of the gastric balloon now being used is achieved by a covering of silk netting material[5] and the specimens obtained have been in most cases very satisfactory from the point of view of preservation as well as an increase in the number of exfoliated cells.

Careful preparation of the patient prior to the test is extremely important as the stomach must be as free as possible of all foreign material. Food, barium, oil, etc., interfere with cytologic interpretation. The patient is given a liquid evening meal and no food, liquids or medication by mouth after midnight. In cases of gastric retention, a three day liquid diet may be necessary with gastric lavage the night before the test. Gastric specimens should not be collected for at least three days after a gastrointestinal series, because of the possible retention of barium. The technic for collecting the specimen is as follows:

1. Pass the balloon, (deflated and wet with Ringer's solution) orally, with the patient sitting upright, all dentures having been removed.
2. Aspirate and discard the gastric contents unless needed for routine gastric analysis.
3. Lavage the stomach until clean and dry, using up to 500 cc. Ringer's solution.
4. Inflate the balloon by 14-16 compressions of the hand bulb, and withdraw it gently from the pylorus to the cardia where slight resistance is felt, usually accompanied by a gag reflex.
5. Allow peristalsis to carry the inflated balloon back to the pylorus.
6. Deflate briefly to allow further passage into the antrum.
7. Re-inflate and draw balloon again up to the cardia.
8. Repeat this procedure 4-5 times, over a period of 30 minutes.
9. Aspirate gastric contents during the above procedure; save a small representative portion of each aspirate and mix it with 95% alcohol.
10. Deflate balloon and withdraw it.
11. Rinse balloon in Ringer's solution and 80% alcohol, equal parts.

Occasionally, gross pieces of tissue will be found clinging to the netting when the balloon is withdrawn from the stomach. This material is removed by means of forceps and may be either smeared directly on slides and fixed in ether-alcohol, or added to the Ringer's solution in which the balloon is to be rinsed. The preserved portions of the gastric aspirate which were obtained during the test may be pooled with the balloon rinsings, or the two specimens may be processed separately and smears pre-

pared from each. An evaluation of the comparative merits of the three types of material obtained with the balloon, i.e. direct smears of gross material, balloon rinsings and gastric aspirates has shown that the rinsings generally contain a large number of better preserved cells.

The laboratory processing of gastric specimens and the preparation and staining of smears are the same as for other types of fluids as previously described. However, prompt handling of the material is essential because of the rapid degeneration of gastric cells. If the entire laboratory procedure cannot be followed through without interruption, it may be temporarily discontinued at either of two points without causing too much damage to the specimen. It should be centrifuged with as little delay as possible, and, after centrifugation, the supernatant liquid decanted, and the sediment covered with a few cubic centimeters of absolute alcohol. It can then be placed in the refrigerator for a short time if smears cannot be prepared immediately. After smears have been prepared and placed in ether-alcohol, they may be left in the fixing solution for several hours or even a few days before being stained.

The rate of exfoliation of cells from the gastric mucosa in normal, nonpathologic cases is not high, but the mildly abrasive action of the balloon as it rubs against the lining of the stomach can increase the exfoliation. Fair sized clusters of cells or even small fragments of tissue may be found in the smears and are usually very well preserved. If a malignant neoplasm is present, clusters of cells may in like manner be freed from the tumor's surface by the action of the balloon. The nuclear structure of these cells is in most cases distinct and well stained, a very important factor in their evaluation, since most of the criteria of malignancy are based on characteristics of the nuclei.

Occasionally a gastric balloon specimen will show poor cellular preservation because of improper cleansing of the stomach. In general, however, the results obtained by using the balloon technic are far superior to those with aspiration or lavage specimens.

An analysis of the cytologic reports in cases of proven carcinoma of the stomach has shown the following accuracy: of the cases in which gastric *aspirates* were examined, 51% were reported as suspicious or positive for a malignant neoplasm; of those with gastric *lavage* specimens, 68% were suspicious or positive;[6] of those with *gastric balloon* specimens, 88% were suspicious or positive.[5] Additional, but as yet incomplete statistical studies of positive cases in which gastric balloon specimens were examined, show that the accuracy may be even higher than 88%.

It is not necessary for a physician to collect the gastric balloon specimen. A technician can be trained to do that and also the

laboratory procedures and the preliminary microscopic examination of the smears. Although the apparatus looks as though it would be disagreeable to swallow, very little difficulty has been encountered in persuading patients to do so. Contra-indications for its use are cirrhosis of the liver, esophageal varices and recent gastric hemorrhage. In the latter case, a balloon may usually be passed after an interval of a week or ten days. As for the danger of hemorrhage being caused by the action of the balloon, nearly 500 specimens have been collected by technicians of the Papanicolaou Cytology Laboratory and so far there have been no ill effects.

Other methods for the diagnosis of carcinoma of the stomach have been developed elsewhere, two of which might be mentioned briefly. Rosenthal and Traut[7] of the University of California use Papain for a mucolytic agent as an aid in concentrating the cells. Insufficient trial has been given this method in our laboratory, but from the specimens examined one thing was noted. By dissolving the mucus, groups of tumor cells were dispersed so that single malignant cells were present for the most part. This seemed to us to make diagnosis more difficult since several important criteria of malignancy may be found in clusters of cells. Rubin and his associates[8] of the University of Chicago have recently modified the abrasive gastric balloon, substituting a mercury weight for the Rehfuss bucket tip in order to carry the end of the tube into the duodenum. They claim very good results from its use.

The methods which have been described here for improving the quality of cytologic smears should by no means be considered the final solution to all problems of exfoliative cytology. There are still many phases of the technic which could be improved and it is to be hoped that, with so many laboratories taking an active interest in the subject, other procedures will be developed.

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PRACTICAL CONSIDERATIONS ON THE USE OF RADIOACTIVE IODINE (I-131) IN THYROID DISORDERS

ROBERT W. WILLMOTT, M.S.
Staff Physicist, Good Samaritan Hospital, Lexington, Kentucky

Radioactive Iodine, through its thirteen years of known existence, has become firmly entrenched as a valuable diagnostic and therapeutic agent in the hands of the practicing physician and surgeon. Its use has now gained the acceptance of the American Medical Association in both of these purposes. To describe this drug, we find that to all outward appearances we have exactly the same element as ordinary iodine or I-127. We note no differences in its chemical properties. An important difference we do find, however, is that radiant energy emanates from the nuclei of our I-131 atoms. It is with this energy we must deal, and the handling of such an odd product gives rise to new and different laboratory techniques. Since the drug is, at present, only produced in large quantities through neutron bombardment, it is the role of the Atomic Energy Commission to both manufacture this radioisotope and insure its distribution to organizations qualified and equipped to properly disseminate it to the public.

The thyroid gland has long been known to concentrate iodine in considerably larger quantities than any other tissue of the body. In the normal gland the concentration by weight is approximately 2000 to 1 over other tissue. In the hyperplastic thyroid this concentration may exceed 10,000 to 1. We find that this very property of iodine collection is indicative of the activity of the gland in its production of thyroxine. Of course, the limitation of iodine saturation must not be exceeded. By the use of a very small quantity of radioactive iodine such a measurement is not only accurate, it is extremely practical and easy. Now, consider the problem of measuring thyroid activity. Shall we administer it orally or intravenously? Both methods have received extensive use in the past and it is well established that an oral administration is much less cumbersome and far less demanding on trained personnel. The prime advantage of an I.V. use is that the thyroid receives the iodine much more rapidly and thus the time of the test can be shortened. How much of the drug shall be given for this thyroid test? The prime determinate is that we are using radiant energy internally and must use amounts which are physiologically inert. Thus the smallest amount accurately measureable which will give good statistical results without effect on the patient is desirable. Equipment in general imposes this limitation with scintillation counters effectively handling amounts of 5-10 microcuries, bismuth cathode geiger tubes handling 10-50 microcuries, and standard geiger tubes handling 50-100 microcuries. The distance over which the meas-

urement is made is of prime concern. Distances in common practice are from 5-20 inches. Shorter distances lead to enlarged errors through positioning of patients and possibility of including a large fraction of beta rays in the reading, even with a properly screened tube. Larger distances lead to either smaller readings making statistical errors great, or to larger doses.

To examine the possible physiologic effect we can say that a 100 microcurie source of radiant energy administered internally is below any known threshold level and the amount of iodine employed in such a dose is of the order of a thousandth of a microgram, or about one fifty-thousandth of the normal daily adult requirement.

Before performing an iodine uptake on a patient, it is of foremost importance to determine if contraindications exist for the test. These consist of a recent history of iodine intake on the part of the patient. Simple iodine as an expectorant, or in thyroid therapeutic amounts, or iodinated thiourea compounds and even the iodinated dyes used in gall bladder and bronchial studies, constitute sufficient reason for postponement of the test. Clearance rates for these agents may be safely taken as follows: one week for iodine in less than thyroid therapeutic amounts, two weeks from therapeutic amounts and two weeks from iodinated thiourea derivatives. The dyes used for X-ray studies often will cause interference as long as six months after their use. Assuming no contraindications do exist, then the patient is placed on the examining table with a low pillow, so that the neck is horizontal and well exposed. The thyroid is palpated and the geiger or scintillation tube placed a set distance from the gland. A series of background counts, usually three, are made over the patient mainly to accustom the patient to the procedure twenty-four hours previous to the actual necessity of such a measurement, and to obtain the background reading.

It is usually convenient to have prepared a solution of I-131 diluted down to contain roughly 50 microcuries per cc. An amount containing the desired radiation can easily be withdrawn with a syringe and placed in a container whose volume approaches the estimated mass of the thyroid gland. Volumetric flasks of 10, 25, 50, and 100 cc provide an adequate answer. An amount of distilled water is added to bring the level of liquid to the bottom of the neck of the flask. A beaker of sufficient size to allow a thickness of water of ½ to 1 inch between its side and the side of the submerged bulb of the flask, is used to represent the radiation absorption of the facia and the epidermis over the thyroid gland. Many more elaborate methods have been used, such as a representative blown glass thyroid placed in a wax neck of normal dimensions, but it has been observed the flask-beaker method gives excellent, consistant, results and cer-

tainly has the advantage of simplicity. It is wise from a statistical viewpoint to then make three separate one minute counts of the diagnostic dose while in the apparatus. The distance one uses is quite naturally the same as that used in the previous measurement of the thyroid. The energy content in terms of counts per minute is recorded and the I-131 solution is poured into a pyrex glass, then the flask is washed, with the wash water also being added to the contents of the glass. The patient then drinks this solution and follows with another drink of distilled water from the same vessel. The patient is then cautioned to remain iodine free in both diet and drugs for a period of 24 hours. No further restrictions on the activities of the patient have been found to be necessary.

After 24 hours have elapsed, the counter is again positioned over the thyroid gland in the same fashion as on the preceding day. Three separate one minute readings will provide good statistical results. There now remains only to calculate the percentage of initial diagnostic dose now residing in the thyroid gland, taking into account the 8% decay of the I-131 during the elapsed 24 hours and the background reading. The procedure just described provides an adequate test for determining simply the activity of the gland. If further information is sought then slight variations of this technique may become necessary. One which is often of value is to determine the urinary output of I-131 as well as the thyroid uptake. The sum of these two percentages should very nearly equal the total amount given. Unaccounted for amounts exceeding 15% may well suggest a metastatic lesion from the thyroid or perhaps a renal difficulty. The content of I-131 in fecal material has not been found informative since only slight traces appear even in myxedematous patients.

Some organizations have found that for their purposes a much speedier iodine uptake determination has been advantageous. These methods center around the rate of iodine absorption through given increments of time, often ten minute periods. The various rate differences give a relationship to the activity of the gland. Most organizations, prefer the single 24 hour determination, whose advantage lies chiefly in the fact that thousands of normals are known, patient handling is faciliated, and results have been excellent.

The radioactive iodine measurement may now be interpreted in terms of our known levels of uptakes as follows:

1. 0- 10%, myxedema
2. 10- 20%, mild hypothyroid
3. 20- 40%, normal, with mean of 33%
4. 40- 60%, mild hyperthyroid
5. 60-100%, toxic hyperthyroid

It must be clear this test does not measure hyperthyroidism as

such directly, but measures the avidity of the thyroid gland for iodine. In order for this measurement to be proportional to the production of thyroxine such conditions as iodine starvation, iodine blockage, increased effects from injected thyrotropic hormone, increased effects from action of anti-thyroid drugs, decreased effects from thyroid feeding and with certain pituitary tumors, must not exist.

From a radiologic safety standpoint, the diagnostic dose does not require lead shielding or remote handling equipment. The time of close contact with the material can be kept to a minimum and precautions to prevent spillage on the hands should be used. Under these conditions, approximately 1 milliroentgen per test would fall on the hands of the operator with considerably less on the body. Unless a very large number of tests per week were being accomplished, the maximum allowable weekly dose of 300 milliroentgens would not be approached.

Therapeutically, radioactive iodine has shown its most remarkable results in the treatment of hyperthyroidism of Graves Disease. Selection of patients for treatment by the clinician is of extreme importance since the diagnostic test does not differentiate between Graves Disease and toxic, nodular goiter or between non-toxic, nodular goiter and malignant nodules. Much has been published on the selection of patients and the results of treatment of the various thyroid disorders.

Shielding is a partial solution to the handling problem of therapeutic amounts of I-131, but distance and time control are still more important considerations. Radioactive iodine radiates both beta rays and gamma rays. The beta's constitute the most destructive in tissue and make up about 85% of the total radiation present. Their range from an unshielded source is approximately 1 meter in air. Roughly two-thirds of the beta's are dissapated in 2 millimeters of tissue or in 1 millimeter of lead. Protection from these particles is not a difficult problem. The rubber gloves, the glassware, and a minor amount of lead shielding give adequate protection. The remaining 15% of the radiation or the gamma rays form the real hazard in the handling of radioactive iodine. A source of 25 millicuries, through a glass syringe surrounded by 2 millimeters of lead gives off at 12 inches nearly 25 milliroentgens per hour of gamma energy. Larger amounts give off correspondingly larger amounts of gamma radiation. Without application of a proper technique, it is difficult to maintain shielding and distance and thus give adequate protection to the operator.

Storage and handling of radioactive iodine can be made considerable less expensive by selection of a suitable working area. A small concrete closet in the basement furnishes a good solution since three of the lateral sides are enclosed with a concrete

barrier. The floor presents no problem and the overhead is usually of sufficient distance and material that further protection is not necessary. If a closet is not available, then an outside corner may be employed, with only the addition of a small lead brick wall. A strong work bench is placed in this area and provision made for shielding the operators feet and legs. A lead floor and wall placed on the working surface can be made from commercially available lead bricks. The wall directly fronting the operator should be tall enough to provide adequate screening from the drug, yet low enough to be observed over when necessary. At either side of this wall should be vacant spaces for the arms of the operator and also for the passage in and out of materials. The procedure is as follows: The shipping container is placed behind the lead wall and opened. The shielding of the shipping container is removed with tongs or remote fingers of suitable length for the amount of radiation present. An appropriate sized syringe fitted with a needle long enough to reach the bottom of the bottle, but without plunger, is then inserted through the stopper of the bottle with the remote fingers. A second needle, of shorter length, fitted to a large screw type syringe by a length of rubber tubing, provides a method of introducing sufficient amounts of air pressure to lift the radioactive solution remotely into the first syringe. A remote pipetting device allows the transferral of a measured amount of the solution to a drinking vessel. The patient can then retrieve the vessel and the treatment may be accomplished at this point, or a lead cylinder, chambered to fit the vessel with top and carrying facilities, can be employed if the patient must be some distance from the laboratory. Such a method uses moderately priced equipment, provides speedy handling of the material, and permits maximum doses to be given without appreciable irradiation of the operator.

In general practice is the rule that patients shall be hospitalized when their treatment dose equals or exceeds 30 millicuries. It is also necessary to collect radioactive urine following such high level treatment. The problems herein imposed are many; nursing care of the patient, radiation received by nurses, collection of urine, disposal of urine, radiation given nearby patients, and finally determining when the patient may be discharged from the hospital.

A practical solution to these problems lies in the use of a small portable geiger counter, calibrated in milliroentgens per hour, a set of film badges, and a urine handling and storage technique. At the time of the treatment the volume surrounding the patient can be surveyed and instructions written for the nursing personnel regarding distances and total time for each to spend with the patient. It will usually be found that the allow-

able time is considerably greater than the time normally spent with any patient, if the waste disposal is handled otherwise. All personnel in the area who might contact the patient should be required to wear film badges. This procedure not only supplies an accurate record of health-safety control, but may well preclude legal entanglement. With adequate instructions the handling of the urine can be left entirely to the patient. They may pour directly from their urinal into a lead surrounded pint jar with a sealing lid. This small container may be stored in a concrete closet, or lead lined box on the roof, etc., and the patient again furnished with the same facilities. Total urine collection usually will amount to about two quarts before the radiation is of such a level that plumbing facilities may be used. Since the radioactive iodine uptake was measured prior to the administration of the treatment dose, and since the number of iodine atoms in a treatment dose do not saturate the iodine capabilities of the thyroid gland, the same proportions as found for the uptake measurement will hold for the therapeutic dose. Thus a patient with a 50% iodine uptake given a treatment dose of 100 millicuries will retain 50 millicuries and excrete 50 millicuries during the first 24 hours. After the first 24 hours have passed no appreciable amount of radioactivity will be found in the excretion and bathroom privileges are desirable. Nearby patients should be separated to such a distance as to receive no more than 1¾ milliroentgens per hour. Their safety is thus assured. Discharge of the patient, in general, is calculated upon the basis of the uptake measurement and the decay of the radioactivity. Our example case of 100 millicuries administered to a 50% iodine uptake patient would then require a maximum hospitalization of 6 days. A more practical method of determining the level of the patient's radiation is to have calibrated the portable geiger counter at a set distance with a 30 millicurie source. It is found that often a day or two saving to the patient can be realized since other factors enter in the removal of the material from the patient.

Spillage, waste disposal, and handling of dirty glassware constitute the remaining factors in handling therapeutic doses of radioactive iodine. Spillage on an individual requires an immediate washing and affected clothing discarded and stored until decay of the active solution is complete. Spillage on floors, counter tops, etc., should first be surveyed with the portable instrument. If measurement shows the area is too hot to approach, even for a short time, then decay must be allowed to bring the level down to one in which long handled mops and blotting material can safely be employed. The mop and material so used can easily be placed in the laboratory waste disposal for radioactive material. All radioactive waste from the laboratory must be stored in isolated waste cans, secure from

city collection facilities, and held until decay has brought the level of radiation down to a negligible quantity by actual measurement. The glassware can be rinsed at the time of use into the vessel going to the patient. This vessel can then in turn be rinsed into the patient. The amount of radiation remaining in the glassware is slight and does not prevent its re-use as long as only radioactive iodine is employed.

Thus several practical considerations for handling radioactive iodine have been presented. Its use as a diagnostic agent is dependent upon its radiation characteristics and the physiologic concentration of iodine by the thyroid gland. Such a diagnostic test involves a very small amount of material, a simple, speedy procedure, and results in an accurate thyroid activity determination. Therapeutically its use depends upon the tissue ionizing beta radiation and the iodine concentrating action of the thyroid gland. Large doses may be safely handled by application of techniques designed to protect all personnel from the ionizing radiation of this drug.

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MEDICAL TECHNOLOGY IN HAWAII

I. L. TILDEN, M.D.
Pathologist, Straub Clinic, Honolulu, Hawaii

The practice of Medical Technology is carried on in the Territory of Hawaii much as it is in other parts of the United States. We have an active local chapter of the American Society of Medical Technologists consisting of approximately forty members. This was organized in 1950. Interest and enthusiasm has remained high and I believe this organization is here to stay.

No Shortage of Technologists. Hawaii is perhaps the only part of the United States where a shortage of trained medical technologists does not exist. This is due in large part to the training program offered by the University of Hawaii in conjunction with four approved hospitals in Honolulu leading to a bachelor of science degree in Medical Technology. The students spend three academic years at the University and a fourth year or twelve months in one of the four hospitals. The degree is granted by the University after completion of the fourth year. With present facilities a maximum of eighteen students can be handled annually. This just about takes care of local needs.

In addition, Hawaii is regarded as a desirable place to live by many because of its pleasant, even, year round subtropical climate, and as a result there is a considerable influx of trained people from the mainland United States. In recent years such people have had a difficult time finding positions.

No Short Cut to Proficiency. Every year a number of local highschool graduates are attracted to certain mainland schools which advertise in the local newspapers, and such students find it virtually impossible to find jobs when they return after a year's course of study. You as technologists and we as pathologists should do everything in our power to discourage highschool graduates from entering these schools. There is no short cut to proficiency in this exacting profession.

Living and Working Conditions. Living and working conditions are pleasant in Hawaii, but living expenses are high and salaries are not especially good. Generally speaking, salaries here are somewhat less than on the west coast, but are higher than in some parts of the middle west and the east.

Types of Diseases. Medically speaking we have much the same diseases here that you do on the mainland. Parasitology, contrary to general belief, does not constitute a very large part of our activities. There is no malaria here and amebic dysentery is rare. Taenia saginata is a common infestation in Filipinos on sugar plantations who eat poorly cooked or uncooked beef. Recently human infestation by Fasciola gigantica has been discovered on the island of Hawaii by Dr. Stemmermann and

Alicata. It is acquired by eating infected water cress. Filariasis and schistosomiasis are not acquired or transmitted here although an occasional person from Samoa will show microfilaria in his blood. Leprosy is present, but is declining, and new cases are few in number.

The complexity of our population of around 500,000, which is made up of many racial groups, provides interesting peculiarities in the incidence of disease, particularly neoplastic disease. Filipinos have a relatively high incidence of primary liver cancer, the Japanese a high incidence of gastric cancer, the Chinese a high incidence of nasopharyngeal cancer and Caucasian women a high incidence of breast cancer as compared with women of oriental races.

Desire for Statehood. Although far removed from medical technology, I can not resist getting in a plug for statehood. Living in Hawaii is not much different than living elsewhere in the United States. We pay the same federal taxes that you do and would like to be admitted as the 49th state so that we can vote for our governor, and for president, and have representation in congress. Taxation without representation seems unfair to us.

EDITORIAL

To all Registered Medical Technologists (ASCP):

No one will disagree with the fact that many individual inequalities, both financial considerations and professional recognition, exist throughout the country. But at the same time we all forget the many instances where technologists have made great progress in improving working conditions and salaries and in establishing professional recognition and creative jobs. There are countless opportunities today for the technologist who has the ability, the initiative, the unselfishness, and the vision to grow with and contribute to the practice of Medical Technology. We all take for granted the accomplishments that have been made in improved teaching laboratories in establishing certification, and in our professional growth.

What many of us forget also is that Medical Technology is a young profession and that our profession started on a haphazard basis with no established standards for minimum requirements of education, clinical training, or classification of laboratory personnel with proper financial rewards. To add to this confusion, the production of adequately trained technologists has in no measure kept pace with the tremendous growth of laboratory science. Consequently we are now in a difficult and restless period of growth during which solutions for the many problems are being impatiently demanded. But perfect and ideal solutions do not emerge quickly just because we want them. There must be a steady growth with prudent planning for sound educational standards, dynamic public relations programs, and adequate provision for career jobs. All this takes time. It takes patience. It takes understanding. It takes tolerance. But above all it needs the help and support of every graduate technologist in this country to build a profession unified by our conduct and achievements into one worthy of the respect of our colleagues in medicine as well as the people we serve.

The A.S.M.T. now has representation on both the Board of Registry and the Board of Approved Schools of the American Society of Clinical Pathologists. We are in the challenging and stimulating position of actually helping to plan our future and to realize some of our aims. Every qualified technologist should have enough pride and faith in his chosen vocation to want to become registered and to support local and national organizations in this effort to make the profession of Medical Technology what we hope and know it can be.

Lastly permit me to add a word of caution about establishing rules and regulations that cannot possibly be enforced by the very fact that the number of properly qualified people are not available for all the jobs. However, do not let this deter us from constantly working toward our aims and aspirations just because everything cannot be set right immediately. No amount of legislation and lobbying can produce Medical Technologists. Nor can legislation produce professional recognition. This is in our hands.

Ruth Hovde, President-elect
ASMT

BOOK REVIEW

REVIEW OF PHYSIOLOGICAL CHEMISTRY, 4th Edition: by Harold A. Harper, Ph. D., Professor of Biochemistry, University of San Francisco, Biochemist Consultant to Metabolic Research Facility, U. S. Naval Hospital, Oakland and to St. Mary's Hospital, San Francisco; Lecturer in Surgery, University of California School of Medicine, San Francisco. Lange Medical Publications, Los Altos, California, 1953. 319 pp. 28 tables. 17 diagrams. $4.00.

This book cannot take the place of a good textbook of Physiological Chemistry, but is designed to supplement such a text in much the same manner that a series of lecture notes does. The material covered is up-to-date and the presentation is in a concise clear manner. The index is very detailed and an excellent list of reference books is included.

The book should be useful to those who wish to review the subject for it enables one to discover in a relatively short time in what areas of the field of physiological chemistry ones knowledge is deficient. Then he can proceed to the more extensive texts for those subjects.

E. Freier

ANNOUNCEMENTS

COURSE IN EXFOLIATIVE CYTOLOGY

Announcing the next course to be given in Exfoliative Cytology at Cornell University Medical College, Miss Charlotte Street of the Papanicolaou Cytology Laboratory says that a three month intensive course will be given from March 1 to May 29, 1954. The program includes lectures, demonstrations, discussions, practice in the preparation and staining of smears, and practical instruction in the interpretation of cytologic material. The course is open to selected physicians and technologists. A certificate is given to those who attend a complete course and demonstrate progress satisfactory to the laboratory staff. For individuals unable to take the three month spring course, training may be obtained on an informal basis throughout the year except for the months of July and August. Instruction for the spring course will be given by Dr. George N. Papanicolaou, Dr. C. N. Foote, Dr. John F. Seybolt, Dr. Irena Koprowski, Miss Charlotte Street and other members of the staff. Further information may be had by writing to Miss Charlotte Street (technologists) or to John F. Seybolt, M.D. (physicians), Papanicolaou Cytology Laboratory, Cornell University Medical College, 1300 York Avenue, New York City.

CONVENTION NOTES

Dear Member:

The annual A.S.M.T. meeting will be in Miami Beach. Do come and see the light, both from the sun and from the talks and workshops presented. We believe that we have worked out the proper amount of each for you so that this trip will be not only profitable, but enjoyable to you. For the comfort of those of you who have never been to Florida at this time of year, the temperature is 80° (average of past 5 yrs.). Casual cottons are worn for all day time events except for the beach where bathing suits and shorts are the accepted thing. (For men slacks and sport shirts are usual.) Do take our advice and be wise in the amount of time spent in the sun. The evenings are often cool enough for a light jacket.

Advance notice from the Program Committee; Speakers already scheduled for the Miami Beach Convention are:

Dr. Lall G. Montgomery, chairman, Board of Registry.
Dr. H. W. Brown, Professor of Parasitology, Columbia University
Dr. G. V. Squires, Pathologist—Pensacola
Dr. C. B. Pollard, Organic Chemist—University of Fla.—Gainesville.
Dr. Sherman Kaplan, Hematologist, Miami Beach
Dr. John B. Miale, Professor of Clinical Pathology—University of Miami Medical School.
Dr. Homer Marsh, Professor of Bacteriology—University of Miami Medical School.
Dr. Robert Boucek, Research work, Miami Heart Institute.
Dr. George T. Lewis, Professor Biochemistry, University of Miami Medical School.
Dr. Philipp R. Rezek, Prof. of Pathology, University of Miami and Director of Pathologic Anatomy at Jackson Memorial Hospital, Miami.

The members of the program committee are:

Chairman—Anna Bell Ham, 1190 S. Alhambra Circle, Coral Gables, Fla.
Katherine Dantzler, 114 N. W. 10th St., Gainesville, Fla.
Carolyn Martin, 1201 Clifton Rd., Atlanta, Ga.
Betty Paulson, 515 E. 41st St., Savannah, Ga.
Philip Cash, 2535 1st Ave. North, St. Petersburg, Fla.

Register now and avoid the June rush.

FROM THE PROGRAM COMMITTEE:

The following is the list of awards available:

1. The Hillkowitz award of $200.00 offered by the Denver Chemical Co. The work reported MUST BE ORIGINAL. Only members of ASMT are eligible for this award.
2. The Registry award of $50.00. This award is open to any registered technologist. The work reported does not have to be original.
3. The ASMT awards of $100.00, $50.00, and $25.00. Only ASMT members are eligible, and the work reported does not have to be original.
4. A parasitology award of $25.00 for the best paper on parasitology. Only ASMT members eligible.
5. The ASMT awards of $75.00 and $50.00 for state society and individual member exhibits.

CORRECTION: To compete for the Hillkowitz Award, the work *must be original.* It was erroneously stated in the November journal that the work need *not* be original.

PROGRAM RULES:

1. The deadline date for the receipt of papers is March 15.
2. All competitive papers must be presented at the convention in person (preferably) or by proxy.

3. All papers read at the convention or presented to the program committee become the property of ASMT and may be published in the American Journal of Medical Technology only. Papers previously published elsewhere cannot be accepted. Prize papers from state contests are eligible for further awards if they have not been published.
4. All audio-visual aids and operators will be furnished by the Speakers Supplies Committee.
5. Five (5) copies of each manuscript submitted for an award must be sent to the program chairman. These must be typewritten, double-spaced, on typewriter paper. The form of the bibliography shall be as follows: Author(s) name(s), title of paper, journal in which published, volume, page, year of publication.
6. Two (2) copies of each manuscript must be submitted by those not competing for awards, subject to the above regulations.

ANNA BELL HAM, program chairman
1190 South Alhambra Circle
Coral Gables, Florida

COME TO MIAMI BEACH IN JUNE
(June 13-17, 1954)

In order to facilitate making plans for what we hope is one of the most successful A.S.M.T. conventions, we are presenting a hotel reservation form. The success of the meeting is dependent upon your participation so plan early to attend. All reservations will be made through the housing committee.

The Hotels Delano and Di Lido are joint headquarters. Since everyone cannot be accommodated in these hotels, it would be appreciated if members would arrange to share rooms or indicate a willingness to be assigned a roommate by the housing committee. In case of two persons making reservations together, confirmation will go to the first one making the request. Please do not send any money to the Housing Committee. Be specific about your arrival date.

Date of Arrival:_____

Time: A.M._____ P.M._____ After 6:00 P.M._____

Date of Departure:_____

Name:_____

Address:_____

Since delegates will be placed at the Delano and Di Lido, will you please check which of the following you are:

1) member _____
2) delegate _____
3) advisory council _____
4) board member _____

Please indicate accommodations desired:

Rooms: Single ($8 or $10)_____ Double ($8 or $10)_____

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If no room is available at the rate requested, please check here_____ if the next higher rate will be agreeable.

Special rates are being secured for Sisters, and information will be sent directly to them.

Reservations may be made with the Chairman of Housing,

Miss Helen Partin
5801 N. E. 4th Court
Miami, Florida

In order to facilitate local handling of reservations we would appreciate it if our colored members would contact Mrs. Adele Mason, Jackson Memorial Hospital, Miami, Florida.

The next Journal will have information concerning the various entertainments and their individual costs. For those interested in pre-convention or post-convention travel, write to Mrs. Adele Mason, Jackson Memorial Hospital, Miami, Fla.

MAKING THE MOST OF MIAMI BEACH

The famous moon will be shining at its fullest during the June 13-17 convention dates at Miami Beach. We are planning to take advantage of it with a program made up with what we think (and hope you will think) is just the right mixture of entertainment and science.

The greater Miami area in what is called "America's Tropicland," has much to offer in the way of different and unusual sights. A general tour will be conducted Sunday afternoon, June 13, either by boat, to see the waterfront homes on the Venetian-like islands or by land, to see the beaches, skyline, and beautiful Hialeah Race Course.

The next day is the official opening of the convention and commercial exhibits. This will be followed by a scientific meeting before a cabana luncheon. The afternoon and evening will be given to scientific programs.

Tuesday morning and early afternoon is to be spent in meetings and discussions, but later there will be movies or a trip to the fabulous Deering home, Villa Vizcaya, which holds art treasures collected from all over the world surrounded by formal gardens. Back to workshops at night.

While the House of Delegates meets Wednesday morning, other members may have a choice of any of the aforementioned tours, but there will be workshops at luncheon and meetings for the early afternoon. Work will be forgotten later for swimming and a picnic at one of the many palm-lined beaches of Biscayne Key. Imagine this, all moonlit with the ocean waves rolling in.

There will be meetings all day Thursday with the banquet as the grand finale.

You will receive an advance copy of the entire program early in May. Point your plans toward Miami Beach and the moon in June.

Mrs. Maxine Ace
Mrs. Anna Rundell
Co-Chairmen, Publicity Committee

SCIENTIFIC EXHIBITS

Just a reminder that it is not too early to reserve a booth for your scientific exhibit. Start now to prepare your exhibits for the 1954 A.S.M.T. convention and write Emelia Lanzetta at 3214 Liddy Ave., West Palm Beach, Fla.

OLD JOURNALS NEEDED

The AMERICAN JOURNAL OF MEDICAL TECHNOLOGY needs copies of the first issues of the Bulletin of the American Society of Clinical Laboratory Technicians: Volume 1, Nos. 1 (November 1934) and

1a (January 1935)

2 (March 1935)

5 (September 1935)

as well as copies of THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY: Volume *2*, Nos. 3 (May 1936) and 6 (November 1936) and a limited number of Volume 3, Nos. 3 (May 1937) and 4 (July 1938)

Volume 4, No. 1 (July 1938)

Volume 11, No. 3 (May 1945)

Volume 12, No. 1 (January 1946)

Volume 19, No. 1 (January 1953)

Please write to AJMT, 25 Hermann Professional Bldg., Houston 25, Texas, if you would like to sell your copies at $1.00 each. Do not ship copies until notified by this office.

STATE SOCIETY PUBLICATIONS AND EDITORS

To State Societies of Medical Technologists: If your BULLETIN and Editor are not listed below, please send information to AJMT, 25 Hermann Professional Bldg., Houston 25, Texas.

Alabama, *Newsletter*, William D. Loach, Druid City Hospital, Tuscaloosa
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California, *California Newsletter*, Mrs. Adabelle N. Thomson, Box 641, Corte Madera
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Ohio, *"The Bulletin" O.S.M.T.*, Adah Sutton, 1228 East Main Street, Columbus
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Tennessee, *Tennessee Newsletter*, Rebekah Lambert, 111 Nesper Road, Oak Ridge
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Vermont, *"Tech-Talk,"* Alice Laughlin, Nutrition Research Department, Medical College, University of Vermont, Burlington
Virginia, *Technoscope*, Mrs. Frances Crouch, 3633 Roundhill Avenue, Roanoke
Washington, *Newsletter*, Mrs. Jacqueline Bahrenburg, St. Luke's Hospital, Spokane
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PLEASE SEND US YOUR ADDRESS CHANGE so that you will receive your journal regularly. If you are going on a vacation "between journals" do NOT notify us. An address change authorized by YOU is the most satisfactory. Failure to let us know where you are located may be the reason you miss an occasional issue of the journal.

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"Technologist (with Masters degree or equivalent) to be key man in clinical laboratory employing five to six technologists; 130-bed hospital with large outpatient service; Southern California, 50 miles Los Angeles. Salary $500-$600 per month depending on experience. Apply to Box G, AJMT."

Clinical Laboratory Technicians, male or female. Registry (ASCP) for 500-bed General Hospital. 40-hour week, no night emergency calls. Liberal personnel policies. Salary schedule available on request to interested persons stating qualifications. Contact Personnel Director, St. Vincent's Hospital, 2213 Cherry Street, Toledo 8, Ohio.

Help Wanted: Clinical Laboratory Technologist, salary $2820 per year. For particulars, contact Superintendent, Gardner State Hospital, Gardner, Massachusetts.

Memorial Hospital at Easton, Maryland, on Maryland's beautiful Eastern Shore has openings for two laboratory technologists. Must be certified by American Society Clinical Pathologists. Starting salary $300.00 month plus cash maintenance allowance $23.00 month. 44-hour week arranged to allow periodic long week-ends off. Eighteen days paid vacation annually after 6 months service. Annual merit salary increases up to 3 years. Blue Cross group. If interested call or write E. C. H. Schmidt, M.D., Director of Laboratories, Memorial Hospital, Easton, Maryland, for personal interview, your expenses will be paid.